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(71) Applicant: **DUKE UNIVERSITY** [US/US]; 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US).

(72) Inventors: **CHILKOTI, Ashutosh**; c/o Duke University, 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US). **HEGGESTAD, Jacob**; c/o Duke University, 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US). **BRITTON, Rhett**; c/o Duke University, 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US). **KINNAMON, David**; c/o Duke University, 2812 Erwin Road, Suite 406, Durham, North Carolina 27705 (US).

(74) Agent: **ZERN, Blaine J.** et al.; Michael Best & Friedrich LLP, 790 N. Water Street, Suite 2500, Milwaukee, Wisconsin 53202 (US).

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(54) **Title:** METHODS FOR CONCURRENT SARS-COV-2 STRAIN DETECTION

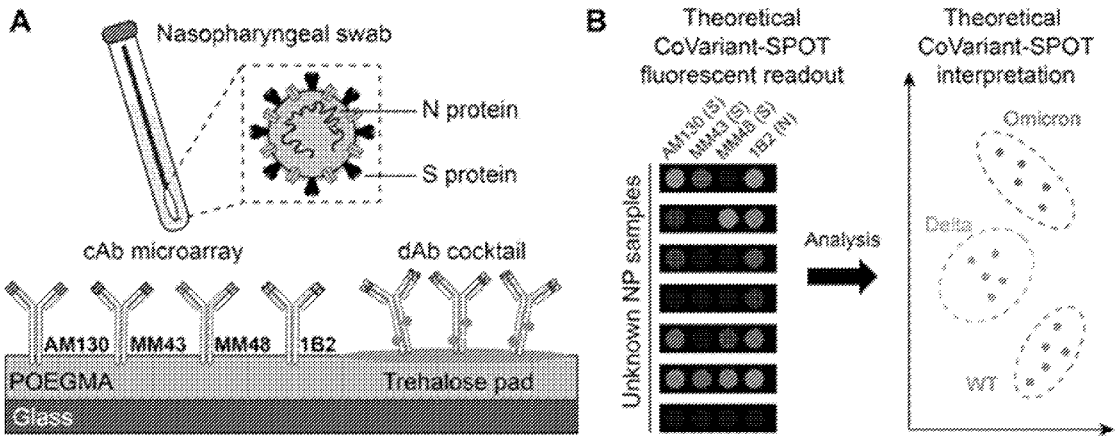

FIG. 1

(57) **Abstract:** Disclosed herein are devices that can be used to detect different SARS-CoV-2 variants in a quick and accurate manner. An example device includes a substrate; a non-fouling layer; a capture region including an array of capture agents, each individual capture agent capable of specifically binding to one or more spike-proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array; and a detection region including a detection agent that is capable of specifically binding to a complex formed between an individual capture agent and the one or more spike-proteins. In addition, an example method includes contacting a biological sample with a device, and detecting the presence or absence of different SARS-CoV-2 variants in the biological sample, wherein the presence or absence of different SARS-CoV-2 variants is detected by measuring a detectable signal provided by each complex formed at each discrete location.

WO 2024/119093 A1

[Continued on next page]

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METHODS FOR CONCURRENT SARS-COV-2 STRAIN DETECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/429,316 filed on December 1, 2022, which is incorporated fully herein by reference.

FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. R01 AI159992 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] This disclosure relates to devices and methods for detecting different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in a biological sample.

INTRODUCTION

[0004] Antigen tests to detect SARS-CoV-2 have emerged as a promising rapid diagnostic method for COVID-19. However, current tests are unable to effectively differentiate between variants of concern (VOCs).

SUMMARY

[0005] In one aspect, disclosed herein are devices including a substrate, a non-fouling layer positioned on the substrate, the non-fouling layer comprising a brush-like polymer, a capture region positioned on the non-fouling layer, the capture region comprising an array of capture agents, each individual capture agent located at a discrete location on the non-fouling layer, wherein each individual capture agent is capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array, and a detection region positioned on the non-fouling layer spatially separated from the capture region, the detection region comprising at least one detection agent and an excipient, wherein the at least one detection agent solubilizes upon contacting the biological sample and is capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins.

[0006] In another aspect, disclosed herein are methods of detecting different SARS-CoV-2 variants, the method including contacting a biological sample with a device as disclosed herein, and detecting the presence or absence of different SARS-CoV-2 variants in the biological sample, wherein the presence or absence of different SARS-CoV-2 variants is detected by measuring a detectable signal provided by each complex formed at each discrete location.

[0007] In another aspect, disclosed herein are methods of detecting different SARS-CoV-2 variants, the method including screening and/or identifying capture agents, detection agents, or both that are capable of specifically binding to one or more spike or nucleocapsid proteins of SARS-CoV-2 variants of interest with a device as disclosed herein; contacting a biological sample with a device as disclosed herein including the identified capture agents, detection agents, or both; and detecting the presence or absence of different SARS-CoV-2 variants in the biological sample, wherein the presence or absence of different SARS-CoV-2 variants is detected by measuring a detectable signal provided by each complex formed at each discrete location.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0009] **FIG. 1** shows a schematic of an example device and method. (A) Schematic of CoVariant-SPOT including 4 capture antibodies (cAbs) and a detection antibody (dAb) cocktail of 2 dAbs printed on a trehalose pad. The primary targets of CoVariant-SPOT are SARS-CoV-2 nucleocapsid (N) and spike (S) proteins from nasopharyngeal (NP) swabs. (B) Cartoon schematic of an example CoVariant-SPOT readout, demonstrating the ability to differentiate variants depending on ratio of the fluorescence intensity at different cAb spots.

[0010] **FIG. 2A** is a set of dose-response curves partitioned by cAb for each SARS-CoV-2 strain spiked into lysis buffer. Each data point represents the average of three replicates with standard deviation (SD) shown. Furthest left point is a blank.

[0011] **FIG. 2B** is a limit of detection (LOD) summary for data presented in FIG. 2A.

[0012] **FIG. 2C** is a set of plots of anti-S cAb ratios to differentiate variants. Numbers in the graph represent the concentration of S1 in ng/mL. The accuracy of differentiation is improved at higher S1 concentrations.

[0013] **FIG. 3** shows analytical validation of CoVariant-SPOT using UV inactivated virus. Dose-response curves for each SARS-CoV-2 variant shown as a function of normalized

intensity (y-axis) versus $TCID_{50}$/mL (x-axis). Isolates were spiked into lysis buffer and incubated for 1 h. Each data point represents the average of three replicates, with SD shown.

[0014] **FIG. 4A** is a plot of raw aggregate data for 1B2 for COVID-19 negative and positive samples. Each data point represents the average intensity of a unique sample, run in duplicate.

[0015] **FIG. 4B** shows ROC analysis for 1B2 in diagnosing COVID-19. At the optimal cut point of 2.72 arbitrary units for N, the sensitivity is 68.4% (95% CI: 57.3% – 77.8%) and the specificity is 96.9% (95% CI: 84.3% – 99.8%). The AUC achieved is 0.87.

[0016] **FIG. 4C** shows correlation of 1B2 intensity compared to viral load, as quantified by RT-PCR.

[0017] **FIG. 4D** is a set of plots of anti-S cAb ratios to differentiate between Delta and Omicron variants for all positive COVID-19 samples with 1B2 intensity greater than 2.72 arbitrary units. At a MM43/MM48 cut point of 0.99, all Delta and Omicron samples are discriminated. Samples with an “x” have not been sequenced but are presumed to be a given variant based on sample collection date.

[0018] **FIG. 4E** shows MM43/MM48 plotted against viral load for Omicron and Delta samples. As viral load increases, discrimination improves. The horizontal dashed line represents the optimal MM43/MM48 cut point and the vertical dashed line represents the viral load cut point corresponding to the 1B2 cut point from FIG. 4C.

[0019] **FIG. 5A** shows a schematic of workflow for point-of-care (POC) testing, where a nasal swab is added to lysis/extraction buffer, sample is added by dropper to the microfluidic cassette which automates the assay. The cassette is then imaged on a D4Scope detector.

[0020] **FIG. 5B** shows a photograph of an example microfluidic cassette. Sample is added at the sample inlet (SI) followed by addition of wash buffer to the wash buffer inlet (WB). CoVariant-SPOT reagents are printed in the reaction chamber (RC) where binding occurs. Inset: detailed view of the RC and zoom in of an example cAb array. The incubation time is governed by the length of the timing channel (TC) which ends at the wicking pad (WP) that pulls sample and wash buffer through the channel, leaving a dry and clean RC for imaging.

[0021] **FIG. 5C** is a set of dose-response curves for recombinant N and S1 proteins spiked into lysis buffer and added to the microfluidic CoVariant-SPOT. Each data point represents the average of three replicates, with SD shown as error bars.

[0022] **FIG. 5D** shows a proof of concept study testing a subset of clinical samples from FIG. 4 on an example microfluidic CoVariant-SPOT. For all positive Delta and Omicron COVID-19 samples with1B2—the N cAb—intensity greater than 2.72 arbitrary units, anti-S cAb ratios are plotted to visually discriminate the two VOCs.

[0023] **FIG. 6A** shows antibodies selected by screening against the S1 domain bind similarly to the S trimer. Like the S1 monomers, an attenuation of signal is observed with MM43 binding to Omicron trimer and MM48 binding to Delta trimer.

[0024] **FIG. 6B** is calculated LODs showing that CoVariant-SPOT is slightly less sensitive to S timer as compared to S1 monomer, but the trends between variants remain similar.

[0025] **FIG. 7** is a set of plots showing the impact of incubation time. An 8-point dose-response for WT SARS-CoV-2 N and S1 protein was tested at various incubation times. Sensitivity to both N and S1 proteins increased with incubation time for all antibodies.

[0026] **FIG. 8** is a plot showing Omicron BA.2 subvariant dose-response on CoVariant-SPOT. CoVariant-SPOT maintains a similar sensitivity to the BA.2 subvariant as compared to the standard sublineage (BA.1). Attenuation of MM43 dose-response is observed.

[0027] **FIG. 9** are plots showing anti-S cAb ratios that can differentiate between WT, Delta, and Omicron variants in UV inactivated viruses. Numbers in the graph represent the concentration of the isolates ($TCID_{50}$/mL). Differentiation improves at higher virus concentrations.

[0028] **FIG. 10** is a plot showing comparison of VTM (Redoxica, VTM-500mL) and Acro Biosystems extraction/lysis buffer (catalog number: LY14) to detect N from WT SARS-CoV-2 isolates (ZeptoMetrix). Each data point represents the average of two replicates, with SD shown. Furthest left data point is a blank.

[0029] **FIG. 11** is an exploded view of an example microfluidic CoVariant-SPOT cassette. (i) Base POEGMA coated glass with inkjet printed reagents. (ii) First adhesive layer that creates the (TC) side walls and includes the profile of the (RC) and (WP). (iii) First acrylic layer that seals the (TC) and gives the (RC) it's depth to accommodate the sample volume. (iv) Second adhesive layer that creates the delay channel for the (WB) that ensures sample has settled into the (RC) prior to wash buffer introduction into the (RC). It also maintains the (RC) profile to ensure an optically transparent line-of-sight for D4Scope imaging. (v) Second acrylic layer that seals off the (RC) and wash delay channel while maintaining access for the (SI) and (WB). (vi) Adhesive backing that attaches the inlet reservoir. (vii) 3D printed inlet reservoir where sample and wash buffer are added. (viii) (WP).

DETAILED DESCRIPTION

[0030] Disclosed herein is a rapid point-of-care test (POCT), which can be referred to as CoVariant-SPOT, that uses a set of capture agents that can be either tolerant or intolerant to

spike protein mutations to identify the likely SARS-CoV-2 strain concurrent with COVID-19 diagnosis using capture agents targeting nucleocapsid protein. All reagents can be incorporated into a portable, multiplexed, and sensitive diagnostic platform built upon a non-fouling polymer brush. To validate CoVariant-SPOT, recombinant SARS-CoV-2 proteins, inactivated viruses, and nasopharyngeal swab samples were used from COVID-19 positive and negative individuals and showed that CoVariant-SPOT can readily distinguish between two VOCs: Delta and Omicron. The disclosed devices and methods can serve as a valuable adjunct to next-generation sequencing (NGS) to rapidly identify variants using a scalable and deployable POCT, thereby enhancing community surveillance efforts worldwide and informing treatment selection.

1. Definitions

[0031] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting. Methods and materials similar or equivalent to those described herein can be used in practice or testing of the disclosed invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

[0032] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0033] The modifier “about” used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). The modifier “about” should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression “from about 2 to about 4” also discloses the range “from 2 to 4.” The term “about” may refer to plus or minus 10% of the indicated number. For example, “about 10%” may indicate a range of 9% to 11%, and “about 1” may mean from 0.9-1.1. Other meanings of “about” may be apparent from the context, such as rounding off, so, for example “about 1” may also mean from 0.5 to 1.4.

[0034] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are contemplated, and for the range 1.5-2, the numbers 1.5, 1.6, 1.7, 1.8, 1.9, and 2 are contemplated.

[0035] The term "antibody," as used herein, refers to a protein substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains, respectively. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, Fv', Fd, Fd', scFv, hsFv fragments, single-chain antibodies, cameloid antibodies, diabodies, and other fragments.

[0036] The terms "biological sample" or "sample," as used herein, refer to any material that is taken from its native or natural state, so as to facilitate any desirable manipulation or further processing and/or modification. A sample or a biological sample can include a cell, a tissue, a fluid (e.g., a biological fluid), a protein (e.g., antibody, enzyme, soluble protein, insoluble protein), a polynucleotide (e.g., RNA, DNA), a membrane preparation, and the like, that can optionally be further isolated and/or purified from its native or natural state. Example biological samples include, but are not limited to, a nasal or nasopharyngeal swab, throat swab, and saliva. A biological sample may be in its natural state or in a modified state by the addition of components such as reagents, or removal of one or more natural constituents.

[0037] The term "limit of detection" or "LOD" is the point at which the measured value is larger than the uncertainty associated with its measurement. The LOD is defined as the lowest concentration at which a compound can be qualitatively identified, while quantification may not be accurate.

[0038] A "protein" or "polypeptide" is a linked sequence of 50 or more amino acids linked by peptide bonds. A peptide is a linked sequence of 2 to 50 amino acids linked by peptide bonds.

The polypeptide and peptide can be natural, synthetic, or a modification or combination of natural and synthetic. Proteins and polypeptides include proteins such as binding proteins, receptors, and antibodies. The terms “polypeptide,” and “protein” are used interchangeably herein. “Primary structure” refers to the amino acid sequence of a particular peptide. “Secondary structure” refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, e.g., enzymatic domains, extracellular domains, transmembrane domains, pore domains, and cytoplasmic tall domains, “Domains” are portions of a polypeptide that form a compact unit of the polypeptide and are typically 15 to 350 amino acids long. Example domains include domains with enzymatic activity or ligand binding activity. Typical domains are made up of sections of lesser organization such as stretches of beta-sheet and alpha-helices. “Tertiary structure” refers to the complete three-dimensional structure of a polypeptide monomer. “Quaternary structure” refers to the three-dimensional structure formed by the noncovalent association of independent tertiary units. A “motif” is a portion of a polypeptide sequence and includes at least two amino acids. A motif may be 2 to 20, 2 to 15, or 2 to 10 amino acids in length, in some embodiments, a motif includes 3, 4, 5, 6, or 7 sequential amino acids. A domain may be comprised of a series of motifs, which may be similar or different.

[0039] The term “small molecule,” as used herein, refers to inorganic or organic compounds having a molecular weight of less than 3,000 daltons.

[0040] The term “specifically binds,” as used herein, is generally meant that a molecule binds to a target molecule when it binds to that target molecule more readily than it would bind to a random, unrelated target.

[0041] The term “region,” as used herein, refers to a defined area on the surface of a material. A region can be identified and bounded by a distinct interface between two materials having different compositions.

[0042] The term “subject” and “patient” are used interchangeably herein and refer to both human and nonhuman animals. The term “nonhuman animals” of the disclosure includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, rats, mice, rabbits, pigs, cows, sheep, goats, horses, dogs, cats, fish, birds, and the like. Typically, the subject is a mammal. A subject also refers to primates (e.g., humans, male or female; infant, adolescent, or adult) or non-human primates. In some embodiments, the subject is a primate. In some embodiments, the subject is a human.

[0043] The term “SARS-CoV-2 variant,” as used herein, generally refers to a variant of the SARS-CoV-2 wild type (WT) virus having a single or multiple nucleotide substitution, addition

and/or deletion or truncation mutation in the viral genome and a corresponding single or multiple amino acid substitutions, addition and/or deletion or truncation in a viral peptide, polypeptide or protein. For example, a SARS-CoV-2 variant can have a single or multiple amino acid substitution of one or more spike proteins. The SARS-CoV-2 variant can be about 80% identical, either via nucleotide sequence or amino acid sequence, to about 99.9% identical to the SARS-CoV-2 WT, such as about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the SARS-CoV-2 WT. In some embodiments, each SARS-CoV-2 variant has at least one mutation of one or more spike proteins that is different from the other SARS-CoV-2 variants.

2. Devices

[0044] Disclosed herein are devices that can be used in methods of detecting different SARS-CoV-2 variants. Generally, devices can include a substrate; a non-fouling layer; a capture region including an array of capture agents – each individual capture agent capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array; and a detection region including at least one detection agent and excipient – the at least one detection agent capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins. Upon contact with a liquid, such as a biological sample, the excipient can be dissolved and the detection agent can be solubilized, which can allow the detection agent to interact with other aspects of the device, such as the capture agents and regions thereof. Accordingly, the device can take advantage of differential binding interactions between the array of capture agents and spike proteins of different SARS-CoV-2 variants to detect the presence or absence of different SARS-CoV-2 variants.

A. Substrate

[0045] The substrate can act as a base of the device and can allow for other layers and/or components to be positioned on its surface. A variety of different substrates can be used in the device and may include any suitable material that allows for the disclosed devices to perform a desired function, e.g., detecting different SARS-CoV-2 variants. Examples include, but are not limited to, metals, metal oxides, alloys, semiconductors, polymers (such as organic polymers in any suitable form including woven, nonwoven, molded, extruded, cast, etc.), silicon, silicon oxide, ceramics, glass, and combinations thereof.

[0046] Example polymers that can be used to form the substrate include, but are not limited to, poly(ethylene) (PE), poly(propylene) (PP), cis and trans isomers of poly(butadiene) (PB), cis

and trans isomers of poly(isoprene), poly(ethylene terephthalate) (PET), polystyrene (PS), polycarbonate (PC), poly(epsilon-caprolactone) (PECL or PCL), poly(methyl methacrylate) (PMMA) and its homologs, poly(methyl acrylate) and its homologs, poly(lactic acid) (PLA), poly(glycolic acid), polyorthoesters, poly(anhydrides), nylon, polyimides, polydimethylsiloxane (PDMS), polybutadiene (PB), polyvinylalcohol (PVA), polyacrylamide and its homologs such as poly(N-isopropyl acrylamide), fluorinated polyacrylate (PFOA), poly(ethylene-butylene) (PEB), polystyrene-acrylonitrile) (SAN), polytetrafluoroethylene (PTFE) and its derivatives, polyolefin plastomers, and combinations and copolymers thereof.

[0047] In some embodiments, the substrate includes a glass, a silicon, a metal oxide, a polymer, or a combination thereof. In some embodiments, the substrate includes a glass, a silicon, a metal oxide, or a polymer. In some embodiments, the substrate includes a glass, a silicon, or a polymer. In some embodiments, the substrate includes a glass. In some embodiments, the substrate is a glass.

B. Non-Fouling Layer

[0048] The device includes a non-fouling layer positioned on the substrate (e.g., on a surface of the substrate). The non-fouling layer can decrease non-specific binding and/or adsorption of non-target analytes to the device. Non-fouling, as used herein with respect to the layer, relates to the inhibition (e.g., reduction or prevention) of growth of an organism as well as to non-specific or adventitious binding interactions between the non-fouling layer and an organism or biomolecule (e.g., cell, protein, nucleotide, etc.).

[0049] The non-fouling property of the layer can be instilled through the inclusion of a brush-like polymer. The hydrophilic nature of the brush-like polymer can allow a droplet of, e.g., saliva to diffuse across the entire non-fouling layer surface to potentially interact with other areas of the device, such as the capture region. Generally, brush-like polymers are formed by the polymerization of monomeric core groups having one or more groups that function to inhibit binding of a biomolecule (e.g., cell, protein, nucleotide, carbohydrate/lipid) coupled thereto. The monomeric core group can be coupled to a protein-resistant head group. In some embodiments, the brush-like polymer includes a monomeric core group and a protein-resistant head group coupled to the monomeric core group.

[0050] Brush-like polymers can be synthesized using radical polymerization techniques, such as catalytic chain transfer polymerization, iniferter mediated polymerization (e.g., photoiniferter mediated polymerization), free radical polymerization, stable free radical mediated polymerization (SFRP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) polymerization. For example, free radical polymerization of

monomers to form brush-like polymers can be carried out in accordance with known techniques, such as described in U.S. Pat. Nos. 6,423,465; 6,413,587; and 6,649,138; U.S. Patent Application Publication No. US 2003/0108879 A1 – all of which are incorporated herein by reference in their entirety, and variations thereof which will be apparent to those skilled in the art. Atom transfer radical polymerization of monomers to form brush-like polymers can also be carried out in accordance with known techniques, such as described in U.S. Pat. Nos. 6,541,580 and 6,512,060; U.S. Patent Application Publication No. US 2003/0185741 A1 - all of which are incorporated herein by reference in their entirety, and variations thereof which will be apparent to those skilled in the art.

[0051] Any suitable core vinyl monomer polymerizable by the processes discussed above can be used, including but not limited to styrenes, acrylonitriles, acetates, acrylates, methacrylates, acrylamides, methacrylamides, vinyl alcohols, vinyl acids, and combinations thereof.

[0052] Protein resistant groups can be hydrophilic head groups or kosmotropes. Examples include, but are not limited to, oligosaccharides, tri(propyl sulfoxide), hydroxyl, glycerol, phosphorylcholine, tri(sarcosine) (Sarc), *N*-acetylpiperazine, betaine, carboxybetaine, sulfobetaine, permethylated sorbitol, hexamethylphosphoramide, an intramolecular zwitterion (for example, $–CH_2N^+(CH_3)_2CH_2CH_2CH_2S0_3$) (ZW), and mannitol.

[0053] Additional examples of kosmotrope protein resistant head groups can include:

$–(OCH_2CH_2)_6OH$;

–O(Mannitol);

$–C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$;

$–N(CH_3)_3$+Cl−/$–SO_3–$Na+(1:1);

–N(CH3)2 +CH2CH2SO3;

–C(O)Pip(NAc) (Pip=piperazinyl);

$–N(CH_3)_2$ +CH_2CO_2;

–O([Glc-α(1,4)-Glc-β(1)−]);

$–C(O)(N(CH_3)CH_2C(O))_3N(CH_3)_2$;

$–N(CH_3)_2^+CH_2CH_2CH_2SO_3^-$;

$–C(O)N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2^-$; or

$–S(O)CH_2CH_2CH_2)_3S(O)CH_3$.

[0054] In some embodiments, a protein resistant head group includes poly(ethylene glycol) (PEG), for example PEG of from 1 to 30 monomeric units, such as 2 to 25 monomeric units, 3 to 20 monomeric units, 4 to 18 monomeric units, or 2 to 15 monomeric units.

[0055] In some embodiments, the non-fouling layer is formed by surface-initiated ATRP (SI-ATRP) of oligo(ethylene glycol)methyl methacrylate (OEGMA) to form a poly(OEGMA) (POEGMA) film. In some embodiments, the non-fouling layer includes a functionalized POEGMA film prepared by copolymerization of a methacrylate and methoxy terminated OEGMA. The POEGMA polymer can be formed in a single step. In some embodiments, the non-fouling layer includes POEGMA.

[0056] In general, the brush molecules formed by the processes described herein (or other processes either known in the art or which will be apparent to those skilled in the art), can be from 2 or 5 up to 100 or 200 nanometers in length, or more, and can be deposited on the surface portion at a density of from 10, 20, or 40 to up to 100, 200 or 500 milligrams per meter, or more. In some embodiments, the non-fouling layer has a thickness of about 2 nm to about 500 nm, such as about 2 nm to about 400 nm, about 5 nm to about 300 nm, about 10 nm to about 250 nm, about 2 nm to about 200 nm, or about 5 nm to about 200 nm. In some embodiments, the non-fouling layer is deposited on the substrate at a density of about 10 mg/m^2 to about 600 mg/m^2, such as about 20 mg/m^2 to about 500 mg/m^2, about 10 mg/m^2 to about 500 mg/m^2, about 20 mg/m^2 to about 400 mg/m^2, or about 10 mg/m^2 to about 400 mg/m^2.

[0057] Prior to deposition of further components onto the non-fouling layer, the substrate with an optional linking layer and non-fouling layer can be dry or at least macroscopically dry (that is, dry to the touch or dry to visual inspection, but retaining bound water or water of hydration in the polymer layer). For example, to enhance immobilization of a capture agent, the non-fouling layer can suitably retain bound water or water of hydration, but not bulk surface water. If the substrate with the optional linking layer and non-fouling layer has been stored in desiccated form, bound water or water of hydration can be reintroduced by quickly exposing the non-fouling layer to water (e.g., by dipping into water) and subsequently blow-drying the surface (e.g., with a nitrogen or argon jet). Alternatively, bound water or water of hydration can be reintroduced by exposing the non-fouling layer to ambient air for a time sufficient for atmospheric water to bind to the polymer layer.

[0058] Further discussion regarding the non-fouling layer, the substrate, the optional linking layer, e.g., between the substrate and the non-fouling layer, and methods of printing molecules onto devices can be found in U.S. Patent No. 9,482,664, which is incorporated by reference herein in its entirety.

C. Capture Region

[0059] The device includes a capture region positioned on the non-fouling layer. The capture region can include an array of capture agents. Each individual capture agent can be located at a

discrete location on the non-fouling layer. In addition, each individual capture agent can be capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity (e.g., K_d) than the other capture agents of the array. Spike proteins are inclusive of fragments, as well as multiples (e.g., dimers, trimers, etc.). As an example, where an array of capture agents that includes three different capture agents with differential affinity to two different SARS-CoV-2 variants – one capture agent can have low affinity to one or more spike proteins of the first SARS-CoV-2 variant and high affinity to one or more spike proteins of the second SARS-CoV-2 variant; one capture agent can have high affinity to one or more spike proteins of the first SARS-CoV-2 variant and low affinity to one or more spike proteins of the second SARS-CoV-2 variant; and one capture agent can have medium affinity to one or more spike proteins of the first SARS-CoV-2 variant and medium affinity to one or more spike proteins of the second SARS-CoV-2 variant. The foregoing is a generalization, and any number of variations of capture agents with differential affinity to a variable number of SARS-CoV-2 variants can be used in the disclosed device and methods thereof.

[0060] By using combinations of different capture agents with differential binding to SARS-CoV-2 variants, it has been found that different SARS-CoV-2 variants can be discerned with a high degree of confidence from a biological sample. The degree of which was an unexpected finding because it was originally unknown that mutations in, e.g., spike proteins, of different SARS-CoV-2 variants would be significant enough to alter binding to different capture agents in a way that could be clearly differentiated in a capture agent array format. However, as disclosed herein, it was surprisingly found that mutations of, e.g., spike proteins, of different SARS-CoV-2 variants altered binding to capture agents was enough to provide unique binding event signatures between different SARS-CoV-2 variants and different capture agents that was pronounced enough to differentiate SARS-CoV-2 variants at high enough antigen titers.

[0061] Each individual capture agent can be a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, or a combination thereof. In some embodiments, each individual capture agent includes a peptide, a protein, or a combination thereof. In some embodiments, each individual capture agent includes a peptide or a protein. In some embodiments, each individual capture agent comprises an antibody or fragment thereof. The antibody can be a monoclonal antibody. Capture agents can be commercially purchased or made through recombinant techniques as known within the art.

[0062] In some embodiments, the array of capture agents comprises capture agents selected from the group consisting of DH1041, DH1042, DH1043, DH111, DH1284, DH1193,

DH1044, DH1047, DH1049, DH1050.1, DH1051, DH1048, DH1054, DH1053, DH1055, LT8010, LT5000, LT4000, S1N-M122, S1N-M130, 40150-D001, 40150-D002, 40150-D003, 40150-D004, 40150-MM43, 40150-MM48, 40592-R001, 40592-R117, 40592-R118, and combinations thereof.

[0063] The array of capture agents can include a varying number of different capture agents. For example, the array of capture agents can include 2 to 30 different capture agents, such as 2 to 25, 2 to 20, 2 to 15, 2 to 10, 5 to 30, 10 to 30, 3 to 20, 3 to 15, 3 to 12, 3 to 10, or 4 to 10. In some embodiments, the array of capture agents includes greater than 2 different capture agents, greater than 3 different capture agents, greater than 4 different capture agents, greater than 5 different capture agents, greater than 6 different capture agents, greater than 7 different capture agents, greater than 8 different capture agents, greater than 9 different capture agents, or greater than 10 different capture agents. In some embodiments, the array of capture agents includes less than 30 different capture agents, less than 25 different capture agents, less than 20 different capture agents, less than 15 different capture agents, less than 14 different capture agents, less than 13 different capture agents, less than 12 different capture agents, less than 11 different capture agents, or less than 10 different capture agents. In the foregoing, different capture agents refer to each individual capture agent being capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array.

[0064] The array of capture agents can be deposited on the non-fouling layer by any suitable technique such as microprinting or microstamping, piezoelectric or other forms of non-contact printing and direct contact quill printing. When the capture agents are printed on to the non-fouling layer, it may be adsorbed onto the non-fouling layer such that it remains bound when the device is exposed to a fluid, such as a biological sample. The brush-like polymer may also provide a protective environment, such that the capture agents remain stable when the device is stored. For example, the brush-like polymer layer may protect the capture agents against degradation, which can allow the device to be stored under ambient conditions.

[0065] The array of capture agents may be printed onto the non-fouling layer to form a capture region. The array of capture agents and capture region thereof can be arranged in any particular manner and can include any desirable shape or pattern such as, for example, spots (e.g., of any general geometric shape), lines, or other suitable patterns that allow for identification of the capture region on the surface of the non-fouling layer and substrate. The array of capture agents can be arranged in a predetermined pattern such that the identity of each capture agent is associated with a specific location on the non-fouling layer. In some

embodiments, the capture agents are spotted on the non-fouling layer as a row of individual spots. This arrangement may provide independent replicates and may improve robustness of the assay. For example, a microarray containing microspots of varying capture agent and/or capture agent density may allow a broader range of capture agent concentrations to fall within the dynamic range of a given detector, and may thereby eliminate the dilution series of tests usually run of a single sample.

[0066] Any suitable array configuration can be used. Example arrays of capture agents include, but are not limited to, 2 x 2, 5 x 5, 10 x 10, 50 x 50, 100 x 100, 1,000 x 1,000, 2 x 20, 20 x 2, and the like. In addition, capture agents can be deposited at discrete locations on the non-fouling layer at varying densities. For example, capture agents can be deposited at densities of 1, 3, 5, 10, 100 or up to 1000 capture agents per cm^2. Modern non-contact arrayers can be used in the deposition step to produce arrays having up to 1,000,000 capture agents per cm^2. For example, using dip-pen nanolithography, arrays with up to 1 billion discrete capture agents per cm^2 can be prepared. In some embodiments, the capture agent is present on the non-fouling layer at about 1 capture agent/cm^2 to about 1,000,000,000 capture agents/cm^2, such as about 1 capture agent/cm^2 to about 1,000,000 capture agents/cm^2, about 1 capture agent/cm^2 to about 500,000 capture agents/cm^2, about 1 capture agent/cm^2 to about 800,000 capture agents/cm^2, about 10 capture agents/cm^2 to about 100,000 capture agents/cm^2, about 10 capture agents/cm^2 to about 50,000 capture agents/cm^2, about 1 capture agent/cm^2 to about 10,000 capture agents/cm^2, or about 1 capture agent/cm^2 to about 1,000 capture agents/cm^2. The device may also include duplicate capture regions, e.g., to provide some redundancy or control.

[0067] The capture region can also include an array of threshold capture agents. Each individual threshold capture agent can be located at a discrete location on the non-fouling layer. In addition, each individual threshold capture agent can be capable of specifically binding to one or more nucleocapsid proteins (which is also inclusive of fragments thereof) of one or more SARS-CoV-2 variants. The array of threshold capture agents can provide a useful diagnostic measure. For example, it has been found that nucleocapsid proteins are better at detecting the general presence of SARS-CoV-2, while spike proteins are better at detecting/differentiating variants of SARS-CoV-2 from each other. Counter to a selection process that can be done for the capture agents that can specifically bind spike proteins, threshold capture agents that can specifically bind nucleocapsid proteins can be selected for their ability to produce a similar diagnostic sensitivity regardless of variant.

[0068] The array of threshold capture agents can also be used as a threshold metric for variant determination. For example, confidence in SARS-CoV-2 variant differentiation can be

increased if the array of threshold capture agents, e.g., fluoresces above a certain specified threshold. Accordingly, the array of threshold capture agents can also be referred to as a control region or array of control capture agents.

[0069] The array of threshold capture agents can include a varying number of different threshold capture agents. For example, the array of threshold capture agents can include 1 to 10 different threshold capture agents, such as 1 to 8, 2 to 7, 2 to 5, 5 to 10, 1 to 4, or 1 to 3. In some embodiments, the array of threshold capture agents includes greater than 1 different threshold capture agent, greater than 2 different threshold capture agents, greater than 3 different threshold capture agents, or greater than 5 different threshold capture agents. In some embodiments, the array of threshold capture agents includes less than 10 different threshold capture agents, less than 9 different threshold capture agents, less than 8 different threshold capture agents, or less than 7 different threshold capture agents.

[0070] The description above for the capture agent as it relates to what it can include (e.g., antibody or fragment thereof), how it is deposited, and configurations of the array can also be applied to the threshold capture agents.

D. Detection Region

[0071] The device includes at least one detection region positioned on the non-fouling layer. In some embodiments, the device includes a plurality of detection regions. The detection region(s) are spatially separated from the capture region on the non-fouling layer. The detection region includes at least one detection agent and an excipient. The detection agent can be non-covalently bound to the non-fouling layer. Upon contact with a fluid such as a biological fluid, buffer, or aqueous solvent, the excipient may dissolve and/or absorb into the non-fouling layer. Accordingly, when exposed to an aqueous fluid such as, for example, a biological sample, the detection agent may be solubilized and released into the sample and may bind to a complex formed between an individual capture agent and a spike protein from one or more SARS-CoV-2 variants in the capture region. The excipient may also further stabilize the detection agent during storage.

[0072] The detection agent can be used to visualize the presence of different SARS-CoV-2 variants through sandwich immunoassay principles. For example, the detection agent can be capable of specifically binding to a complex formed between an individual capture agent and a spike protein of one or more SARS-CoV-2 variants, thereby indicating, e.g., through a detectable signal, that one or more SARS-CoV-2 variants is present in the biological sample. In some embodiments, the detection agent is capable of specifically binding to the one or more spike proteins of a formed complex. In addition, in embodiments that include an array of

threshold capture agents, there can be at least one detection agent that is capable of specifically binding to a complex formed between an individual threshold capture agent and a nucleocapsid protein of one or more SARS-CoV-2 variants, thereby indicating, e.g., through a detectable signal, that one or more SARS-CoV-2 variants is present in the biological sample.

[0073] The detection agent can be a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, or a combination thereof. In some embodiments, the detection agent includes a peptide, a protein, or a combination thereof. In some embodiments, the detection agent includes a peptide or a protein. In some embodiments, the detection agent includes an antibody or fragment thereof. The antibody can be a monoclonal antibody. Detection agents can be commercially purchased or made through recombinant techniques as known within the art.

[0074] In some embodiments, the detection region includes at least one detection agent selected from the group consisting of dAb4, dAb5, dAb6, dAb7, dAb8, dAb11, dAb13, dAb16, dAb17, dAb18, dAb19, dAb20, dAb25, and combinations thereof. Ab# corresponds to Table 2.

[0075] The detection region can include a varying number of different detection agents. For example, the detection region can include 1 to 5 different detection agents, such as 1 to 4, 2 to 3, or 1 to 3. In some embodiments, the detection region includes greater than 1 different detection agent, greater than 2 different detection agents, or greater than 3 different detection agents. In some embodiments, the detection region includes less than 5 different detection agents, less than 4 different detection agents, or less than 3 different detection agents. The different detection agents can be capable of specifically binding to at least one complex formed between an individual capture agent and the one or more spike proteins or capable of specifically binding to at least one complex formed between an individual threshold capture agent and the one or more nucleocapsid proteins.

[0076] The capture agent and the detection agent can be included at different ratios to provide advantageous differential visualization of the presence of different SARS-CoV-2 variants. For example, the device and method thereof can include a ratio of total capture agents (including threshold capture agents if present) to total detection agent (including detection agents that can specifically bind to a complex including a threshold capture agent if present) of 2:1 to 5:1. Example ratios include, but are not limited to, 4:2, 5:2, 6:3, 7:2, 8:2, 8:3, 10:5, 10:2, and 10:3.

[0077] In some embodiments, the capture region includes three individual capture agents and one individual threshold capture agent, and the detection region includes one individual detection agent capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins, and an individual detection agent capable of

specifically binding to a complex formed between the threshold capture agent and the one or more nucleocapsid proteins.

[0078] The detection agent can include a detectable signal or label. The detection signal allows the detection agent to be detected and/or visualized directly or indirectly. The detectable signal can include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, or chemical means. Example detectable signals include, but are not limited to, fluorescent nanoparticles (e.g., quantum dots (Qdots)), metal nanoparticles, (e.g., gold nanoparticles) fluorescent dyes (e.g., CyDyes such as Cy3 or Cy5, fluorescein, texas red, rhodamine, green fluorescent protein, and the like, see, e.g., Molecular Probes, Eugene, Oreg., USA), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, ^{99}Tc, ^{203}Pb, and the like), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), various colorimetric signals, magnetic or paramagnetic signals (e.g., magnetic and/or paramagnetic nanoparticles), spin signals, radio-opaque signals, and the like.

[0079] In some embodiments, the detection signal can be a colorimetric signal, a fluorescent signal, a radioactive signal, a magnetic signal, or an enzymatic signal. In some embodiments, the detection signal is a colorimetric signal, a fluorescent signal, or a radioactive signal. In some embodiments, the detection signal is a colorimetric signal or a fluorescent signal. In some embodiments, the detection signal is a fluorescent signal.

[0080] The excipient is a molecule or a combination of molecules that is selected as to allow for a stable, but non-permanent, association between the detection agent and the non-fouling layer. In some embodiments, the excipient can be partially soluble, substantially soluble or soluble in an aqueous solution (e.g., buffer, water, sample, biological fluid, etc.). In such embodiments, the excipient can be selected from the non-limiting examples of salts, carbohydrates (e.g., sugars, such as glucose, fucose, fructose, maltose and trehalose), polyols (e.g., mannitol, glycerol, ethylene glycol), emulsifiers, water-soluble polymers, and any combination thereof. Such excipients are well known in the art and can be selected based on the interaction between the excipient and detection agent, the excipient and the brush-like polymer, the solubility of the excipient in a particular medium, and any combination of such factors.

[0081] In some embodiments, the excipient includes a salt, a carbohydrate, a polyol, an emulsifier, a water soluble polymer, or a combination thereof. In some embodiments, the excipient includes a salt, a carbohydrate, a water soluble polymer, or a combination thereof. In some embodiments, the excipient includes a salt, a carbohydrate, or a combination thereof. In

some embodiments the excipient includes PEG. In some embodiments, the excipient includes trehalose.

E. Other Elements

[0082] In some embodiments, the device may further include an agent to demarcate a patterned region on the non-fouling layer, such that a fluid (e.g., a biological sample) can remain confined to a specified region on the non-fouling layer such that it contacts the capture region and the detection region. Such an agent may be, for example, a hydrophobic ink printed on the non-fouling layer prior to the deposition of the capture region and the components of the detection region. Alternatively, the agent may be a wax. In other embodiments, the sample may be contained or directed on the device through selection of an appropriate geometry and/or architecture for the substrate, for example, a geometry that allows the sample to diffuse to the regions including the capture region and the components of the detection region. In some embodiments the substrate may include a well, or a series of interconnected wells.

[0083] In some embodiments, the device may further include regions printed with control agents. For example, the capture region can include control regions printed alongside the capture region to verify the activity of the detection agent and to normalize the signal from the detection moiety, such as fluorescence intensities. Control regions can also be used to evaluate binding to SARS-CoV-2 WT.

[0084] The disclosed device can also be adapted to a microfluidics-based device. Further description of the device being adapted to a microfluidics-based device and the resultant structures are disclosed in International Patent Application No. PCT/US2021/046833 (published as WO2022/040495), which is incorporated herein in its entirety by reference.

3. Methods

[0085] Also disclosed herein are methods of detecting different SARS-CoV-2 variants using the disclosed devices. The method takes advantage of the unexpected finding that mutations of, e.g., spike proteins, of different SARS-CoV-2 variants can alter binding of capture agents enough to provide a differential spectrum of binding events between different SARS-CoV-2 variants and different capture agents.

[0086] The method can include contacting a biological sample with a device. Example biological samples include, but are not limited to, a nasal or nasopharyngeal swab, a throat swab, and saliva. The sample can be diluted in a buffer prior to contacting the device. An example buffer includes, but is not limited to, a buffer including pH 7.5 Tris Buffer, a microcide (e.g., ProClin-300), and at least one detergent (e.g., tween-20, triton x-100). Another example buffer is one provided by Acro Biosystems as listed in the Examples. However, in some

embodiments, the sample is undiluted and added directly to the device. In addition, the volume of the biological sample can be about 30 µL to about 1 mL, such as about 30 µL to about 900 µL, about 50 µL to about 1 mL, about 40 µL to about 600 µL, about 30 µL to about 500 µL, or about 50 µL to about 700 µL. In some embodiments, the volume of the biological sample is greater than 30 µL, greater than 40 µL, greater than 50 µL, or greater than 100 µL. In some embodiments, the volume of the biological sample is less than 1 mL, less than 900 µL, less than 800 µL, or less than 700 µL.

[0087] The biological sample can come from a subject. The subject can have a varying exposure to SARS-CoV-2 and variants thereof. The methods disclosed herein can be used to detect which SARS-CoV-2 variant, if any, the subject is positive for. Accordingly, the methods disclosed herein can be used to monitor the emergence and/or immunity of SARS-CoV-2 variants in a population. Moreover, the methods disclosed herein can be used to design a treatment plan for the subject depending on the absence or presence of different SARS-CoV-2 variants. For example, the subject can receive a treatment dependent on the presence or absence of different SARS-CoV-2 variants. In some embodiments, the subject is human.

[0088] The method can be used to detect different SARS-CoV-2 variants in an advantageously fast manner. For example, detecting the presence or absence of different SARS-CoV-2 variants can occur in about 5 minutes to about 1 hour after the biological sample contacts the device, such as about 20 minutes to about 1 hour after the biological sample contacts the device, about 15 minutes to 1 hour after the biological sample contacts the device, about 15 minutes to about 50 minutes after the biological sample contacts the device, about 20 minutes to about 40 minutes after the biological sample contacts the device, or about 15 minutes to about 30 minutes after the biological sample contacts the device. In some embodiments, detecting the presence or absence of different SARS-CoV-2 variants occurs in less than or equal to 1 hour, less than or equal to 55 minutes, less than or equal to 50 minutes, less than or equal to 45 minutes, less than or equal to 40 minutes, less than or equal to 35 minutes, or less than or equal to 30 minutes after the biological sample contacts the device. In some embodiments, detecting the presence or absence of different SARS-CoV-2 variants occurs in greater than or equal to 5 minutes, greater than or equal to 10 minutes, greater than or equal to 15 minutes, or greater than or equal to 20 minutes after the biological sample contacts the device.

[0089] The method is capable of advantageously detecting the presence or the absence of more than one SARS-CoV-2 variant at the same time. For example the method is capable of detecting the presence or the absence of at least two, at least three, at least four, at least five,

or more different SARS-CoV-2 variants simultaneously, such as within 1 second, 5 seconds, 10 seconds, 30 seconds, 1 minute, or 5 minutes from when a first SARS-CoV-2 variant is detected to when the last individual SARS-CoV-2 variant is detected.

[0090] Following exposure of a device described herein to a biological sample (e.g., a biological fluid), a signal from the detection agent may be detected using any suitable method known in the art. Example methods include, but are not limited to, visual detection, fluorescence detection (e.g., fluorescence microscopy), scintillation counting, surface plasmon resonance, ellipsometry, atomic force microscopy, surface acoustic wave device detection, autoradiography, and chemiluminescence. As one of skill in the art will appreciate, the choice of detection method will depend on the specific detection agent employed. In some embodiments, the detection method is fluorescence. In some embodiments, measuring the detectable signal is done visually or with a smart device (e.g., tablet, smart phone, portable fluorescence reader (e.g., D4 scope), etc.). Prior to detection, the method may include a washing step. For example, the device may be washed with a buffer, such as one including a surfactant (e.g., Tween) and phosphate buffered saline.

[0091] The detectable signal can be provided by the detection agent. Accordingly, if a detection agent specifically binds to a complex, e.g., formed by a capture agent and a spike protein, the detection signal will be located at the discrete location that corresponds to the capture agent. Because each capture agent of the array can differentially bind to different SARS-CoV-2 variants, a binding event signature (e.g., as measured by fluorescence) can be provided that corresponds to an individual SARS-CoV-2 variant.

[0092] The method can also include a step of screening and/or identifying capture agents, detection agents, or both that are capable of specifically binding SARS-CoV-2 variants of interest/concern. SARS-CoV-2 variants of interest can be variants specifically assessed for their absence or presence in the biological sample. For example, the method can be used knowing that three variants of interest are currently present in a population, and thus the method can be used to detect between the three different variants in biological samples from the population. Screening and/or identifying capture agents, detection agents, or both can be performed on disclosed devices through, e.g., high-throughput screening techniques as disclosed in the Examples herein. Furthermore, SARS-CoV-2 variants of interest can be identified via NGS.

[0093] The disclosed method can have advantageous sensitivity and specificity. For example, the method can have a lower limit of detection for nucleocapsid of less than or equal to 0.07 ng/mL, less than or equal to 0.05 ng/mL, less than or equal to 0.03 ng/mL, or less than or equal to 0.01 ng/mL. In addition, the method can have a sensitivity of about 65% to about

95%, such as about 65% to about 90%, about 70% to about 95%, or about 75% to about 95%. In some embodiments, the method has a sensitivity of greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, or greater than 90%. In some embodiments, the method has a sensitivity of less than 95%, less than 90%, less than 85%, or less than 80%. The method can also have a specificity of about 90% to about 99.9%. In some embodiments, the method has a specificity of greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, or greater than 95%. Sensitivity and specificity of the method can be measured by techniques known within the art, and which are discussed in more detail in the Examples herein. Detection, specificity, and/or sensitivity can be done with a confidence interval of greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, or greater than 95%. In some embodiments, detection, specificity, and/or sensitivity is done with a confidence interval of 95%.

4. Kits

[0094] Also disclosed herein are kits that can be used for, e.g., detecting different SARS-CoV-2 variants in a biological sample. The kit can include a disclosed device, at least one buffer, and one or more packages, receptacles, delivery devices, labels, or instructions. The kit may also include other reagents to facilitate using the device and methods thereof. The choice of buffers and reagents will depend on the particular application, e.g., setting of the assay (point-of-care, research, clinical), analyte(s) to be assayed, the detection moiety used, etc.

[0095] In addition, the kit may include a packaging configured to contain the device and the buffer. The packaging may be a sealed packaging, such as a sterile sealed packaging. By "sterile" it is meant that there are substantially no microbes (such as fungi, bacteria, viruses, spore forms, etc.). In some embodiments, the packaging may be configured to be sealed, e.g., a water vapor-resistant packaging, optionally under an air-tight and/or vacuum seal.

[0096] Following construction of the device, it can be optionally dried, e.g., by mild desiccation, blow drying, lyophilization, or exposure to ambient air at ambient temperature, for a time sufficient for the article to be dry or at least macroscopically dry. Once the device is dry or at least macroscopically dry, it may be sealed in a container (e.g., such as an impermeable or semipermeable polymeric container) in which it can be stored and shipped to a user. Once sealed in a container, the device may have, in some embodiments, a shelf life of at least 2 to 4 months, or up to 6 months or more, when stored at a temperature of 25° C. (e.g., without loss of more than 20%, 30% or 50% of binding activity).

[0097] The kits may further include instructions for using the device. These instructions may be present in the kits in a variety of forms, one or more of which may be present in the kit. One

form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Another form for the instructions could be a computer readable medium, e.g., computer-readable memory (e.g., flash memory), etc., on which the information has been recorded or stored. Yet another form for the instructions that may be present is a website address which may be used via the Internet to access the information at a removed site. Any convenient means may be present in the kits.

[0098] In some embodiments, the kit includes instructions that correlate the number, intensity, or both of detectable complexes with the presence of different SARS-CoV-2 variants in the sample. In some embodiments, the kit includes a reference card that correlates the number and intensity of detectable complexes with the presence of different SARS-CoV-2 variants in the sample. In some embodiments, the kit further includes a smart device (e.g., tablet, smart phone, D4 scope, etc.).

[0099] The disclosed invention has multiple aspects, illustrated by the following non-limiting examples.

5. Examples

Example 1

COVID-19 diagnosis and SARS-CoV-2 strain identification by a rapid, multiplexed, point-of-care antibody microarray

Materials & Methods

CoVariant-SPOT fabrication

[00100] CoVariant-SPOT employs the technology of the D4 assay as described in Joh, D. Y. et al. Inkjet-printed point-of-care immunoassay on a nanoscale polymer brush enables subpicomolar detection of analytes in blood. Proc Natl Acad Sci U S A 114, E7054-E7062, (2017), which is incorporated by reference herein in its entirety. In brief, glass microscope slides were functionalized with a poly(oligo(ethylene glycol) methyl ether methacrylate (POEGMA) non-fouling brush with a thickness of ~50 nm via surface-initiated atom transfer radical polymerization (SI-ATRP). Next, anti-SARS-CoV-2 N monoclonal antibody (mouse IgG, DHVI, 1B2) and anti-SARS-CoV-2 S monoclonal antibodies (Acro Biosystems, catalog #S1N-M130; Sino Biological, catalog #40591-MM43; Sino Biological, catalog #40591-MM48) were inkjet printed onto the slides using a Scienion sciFLEXARRAYER S12 (Scienion AG). Rows of five ~180 μm diameter capture spots for each anti-SARS-CoV-2 antibody were printed at a

concentration of 1.0 mg/mL. Surrounding the capture spots, twelve 1 mm-diameter trehalose spots were printed using a BioDot AD1520 printer (BioDot Inc.) loaded with a 10% (w/v) trehalose solution (~100 nL drop volume). Next, Alexa Fluor 647 labeled anti-N antibody (human IgG, DHVI, DH1218) and anti-S antibody (Acro Biosystems, catalog # S1N-M122) were mixed and deposited on top of the excipient pads using the BioDot printer at a concentration of 0.02 mg/mL for each antibody. Twenty-four assays with this configuration were printed on each 75.6 x 25.0 x 1.0 mm glass slide in a 3 x 8 array. CoVariant-SPOT assays were stored under vacuum for at least 24 h before use. For testing with clinical samples, Trublock Ultra (Meridian Life Sciences) was also inkjet printed onto CoVariant-SPOT slides at 6.0 mg/mL in 1x PBS with 0.05% sodium azide in order to prevent any potential interference from human anti-mouse antibodies (HAMA).

CoVariant-SPOT testing procedure

[00101] CoVariant-SPOT chips were secured in a 96-well microarray hybridization cassette that separates the chip into 24 separate wells. To perform the assay, 60 µL of sample was added directly to an assay well, covered, and incubated at room temperature for 1 h. After incubation, samples were aspirated and chips were rinsed in wash buffer (0.1% Tween-20 in 1x PBS), dried, and then scanned with an Axon Genepix 4400 tabletop scanner (Molecular Devices LLC). The average fluorescence intensity at each capture spot was quantified using the Genepix Pro 7 analysis software. All fluorescence intensities were log transformed prior to analysis.

[00102] Analytical validation with recombinant SARS-CoV-2 antigens was performed by testing a 15-point dose-response curve in triplicate with SARS-CoV-2 N and S1 protein antigens (N: Acro Biosystems, catalog numbers: NUN-C5227, NUN-C52Hr, NUN-C52Ht; S1: Sino Biological, catalog numbers: 40591-V08H, 40591-V08H23, 40591-V08H41). N and S proteins of the same SARS-CoV-2 variant were mixed and diluted with extraction buffer (Acro Biosystems, catalog number: LY14) to a starting concentration of 300 ng/mL for each antigen. Antigens for S trimer and BA.2 S1 were purchased from Sino Biological. The LOD was calculated as described in Armbruster, D. A. & Pry, T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 29 Suppl 1, S49-52 (2008), which is incorporated by reference herein in its entirety. Log transformed values were used for calculating the cAb ratios shown in **FIG. 2C**.

[00103] Analytical validation with UV inactivated SARS-CoV-2 isolates was performed by testing a 15-point dose-response curve in triplicate. Three different isolates were procured, representing each variant tested: WT isolate USA-WA1/2020 (ZeptoMetrix, catalog number:

0810587UV), Delta (B.1.617.2) isolate USA/PHC658/2021 (ZeptoMetrix, catalog number: 0810624UV), and Omicron (B.1.1.529 BA.1) isolate USA/MD-HP20874/2021 (ZeptoMetrix, catalog number: 0810642UV). Isolates were diluted in extraction buffer to a starting TCID50/mL of 1 x 106 for WT and Delta, and 2 x 105 for Omicron. All fluorescence intensities were log transformed and then normalized by dividing each value by the cAb intensity of a blank sample.

Clinical testing

[00104] Clinical samples were either purchased commercially (Discovery Life Sciences) or from patients identified through the Duke University Health System or the Durham Veterans Affairs Health System and enrolled into the Molecular and Epidemiological Study of Suspected Infection (MESSI; Pro00100241) approved by the Duke Health Institutional Review Board (IRB). Samples were accessed via an exempt protocol (Pro00105331). For the samples collected under the MESSI protocol, flocked NP swabs were added to 3 mL of VTM (Dasky Medical, catalog #: 88-221KC; VWR (BD), catalog #: 10769-896) and frozen at -80 °C until testing by CoVariant-SPOT. In addition, viral load was determined using the methods described in the section Quantitative RT-PCR below. All samples are summarized in Table 1.

Table 1. Clinical Sample Summary

#	Source	Subject ID	Age	Gender	PCR Result	PCR test method	Viral load (copies/mL)	Lineage
1	MESSI	7AC7BA	32	Female	Negative	Method 2	N/A	N/A
2	MESSI	322088	79	Female	Negative	Method 2	N/A	N/A
3	MESSI	025CBE	83	Male	Negative	Method 2	N/A	N/A
4	MESSI	DA8A0E	53	Male	Negative	Method 2	N/A	N/A
5	MESSI	1DEB9C	62	Female	Negative	Method 1	N/A	N/A
6	MESSI	9759E7	70	Male	Negative	Method 1	N/A	N/A
7	MESSI	48298F	17	Male	Negative	Method 1	N/A	N/A
8	MESSI	D1D8AC	60	Male	Negative	Method 1	N/A	N/A
9	MESSI	FF27EC	33	Male	Negative	Method 2	N/A	N/A
10	MESSI	4A2671	47	Male	Negative	Method 2	N/A	N/A
11	MESSI	ADAF6C	46	Male	Negative	Method 2	N/A	N/A
12	MESSI	5217A5	54	Male	Negative	Method 2	N/A	N/A
13	MESSI	D234DB	36	Female	Negative	Method 2	N/A	N/A
14	MESSI	ABF76B	36	Male	Negative	Method 2	N/A	N/A
15	MESSI	D41350	44	Female	Negative	Method 2	N/A	N/A

16	MESSI	D41350	44	Female	Negative	Method 2	N/A	N/A
17	MESSI	00D120	50	Male	Negative	Method 2	N/A	N/A
18	MESSI	E48FF4	53	Male	Negative	Method 2	N/A	N/A
19	MESSI	E24969	56	Male	Negative	Method 2	N/A	N/A
20	MESSI	2D814B	51	Male	Negative	Method 2	N/A	N/A
21	MESSI	CE4D04	19	Male	Negative	Method 2	N/A	N/A
22	MESSI	C421AD	19	Female	Negative	Method 2	N/A	N/A
23	MESSI	3E7475	25	Male	Negative	Method 2	N/A	N/A
24	MESSI	F7190B	42	Female	Negative	Method 2	N/A	N/A
25	MESSI	72BF40	53	Male	Negative	Method 2	N/A	N/A
26	MESSI	8FDB69	42	Male	Negative	Method 2	N/A	N/A
27	MESSI	FEC4E9	47	Male	Negative	Method 2	N/A	N/A
28	MESSI	02608B	56	Male	Negative	Method 2	N/A	N/A
29	MESSI	2566E0	54	Female	Negative	Method 2	N/A	N/A
30	MESSI	B4AA7C	59	Male	Negative	Method 2	N/A	N/A
31	MESSI	ED6824	55	Male	Negative	Method 2	N/A	N/A
32	MESSI	150B4B	40	Male	Negative	Method 2	N/A	N/A
33	DLS	KH21-18559	58	Female	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
34	DLS	KH21-18560	37	Male	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
35	DLS	KH21-18564	55	Male	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
36	DLS	KH21-18561	47	Male	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
37	DLS	KH20-36735	67	Female	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
38	DLS	KH20-12011	60	Male	Positive	Abbott RealTime SARS-CoV-2 (RT)-PCR	N/A	N/A
39	DLS	KH20-62850	65	Female	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
40	DLS	KH20-71092	65	Male	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
41	DLS	KH20-71564	41	Male	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A

42	DLS	KH20-78468	30	Female	Positive	Thermo Fisher	N/A	N/A
43	DLS	KH20-75153	66	Male	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
44	DLS	KH20-61677	81	Female	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
45	DLS	KH20-61634	54	Female	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
46	DLS	KH20-71078	65	Female	Positive	PerkinElmer Applied Biosystems 7500	N/A	N/A
47	MESSI	C2C669	39	Female	Positive	Method 1	1356918328	B.1
48	MESSI	A0F8B9	22	Male	Positive	Method 1	88384	Unassigned
49	MESSI	2812A3	52	Male	Positive	Method 1	11363	Unassigned
50	MESSI	3EA6A0	35	Female	Positive	Method 1	152273099	B.1
51	MESSI	4DFE6A	30	Male	Positive	Method 1	2661	B.1
52	MESSI	AA3F84	19	Male	Positive	Method 2	3989154	B.1.521
53	MESSI	598A88	27	Female	Positive	Method 1	113824	B.1.1.231
54	MESSI	7C2A2A	62	Female	Positive	Method 1	10800000	B.1.1.148
55	MESSI	2F0D5E	59	Male	Positive	Method 1	10400	B.1
56	MESSI	C31FF9	78	Male	Positive	Method 1	12900000	B.1
57	MESSI	78DDF0	65	Female	Positive	Method 1	31200	B.1.1.135
58	MESSI	29EEF5	44	Female	Positive	Method 1	48200	B.1.240
59	MESSI	B63F74	65	Female	Positive	Method 1	100000	B.1.2
60	MESSI	00D120	50	Male	Positive	Method 2	408325	B.1.2
61	MESSI	76366E	69	Female	Positive	Method 2	957199	B.1.2
62	MESSI	FFE532	63	Male	Positive	Method 2	185184356	B.1.2
63	MESSI	3501A9	25	Female	Positive	Method 2	14282316	B.1
64	MESSI	5217A5	54	Male	Positive	Method 2	81313	AY.44
65	MESSI	A8FD53	34	Female	Positive	Method 2	3363873	B.1.2
66	MESSI	B4D0DE	63	Male	Positive	Method 2	48304	B.1.1.207
67	MESSI	7400BF	48	Female	Positive	Method 2	602458286	B.1.2
68	MESSI	B2905A	63	Female	Positive	Method 2	9008908	B.1.1.207
69	MESSI	E60E94	15	Female	Positive	Method 2	5755536	B.1.2
70	MESSI	67B41D	15	Female	Positive	Method 2	276176054	B.1.2
71	MESSI	C795F1	53	Male	Positive	Method 2	4605960	B.1.2
72	MESSI	8CF0CC	33	Female	Positive	Method 2	1217247	B.1.526
73	MESSI	FB394D	14	Male	Positive	Method 2	2714713	B.1.526
74	MESSI	17A783	33	Female	Positive	Method 2	692489	AY.118

75	MESSI	9E259D	31	Male	Positive	Method 1	94408300	AY.118
76	MESSI	9FAB68	66	Male	Positive	Method 1	276926644	AY.103
77	MESSI	E55DCE	31	Male	Positive	Method 1	53113968	AY.103
78	MESSI	EB2A47	54	Male	Positive	Method 2	438627835	AY.44
79	MESSI	85F0AB	51	Male	Positive	Method 2	138749	AY.103
80	MESSI	C9EEEA	37	Male	Positive	Method 2	4991978	AY.44
81	MESSI	ADAF6C	46	Male	Positive	Method 2	200625	AY.44
82	MESSI	3E8F04	35	Male	Positive	Method 2	67287	AY.103
83	MESSI	1E4BD7	44	Male	Positive	Method 2	417455	AY.44
84	MESSI	8655BF	38	Male	Positive	Method 2	8638159	AY.44
85	MESSI	EE435A	48	Male	Positive	Method 2	1600293	AY.103
86	MESSI	990CA3	67	Male	Positive	Method 2	38730392	AY.103
87	MESSI	845B87	58	Male	Positive	Method 2	37300	AY.103
88	MESSI	A97D5B	27	Male	Positive	Method 2	1298723761	AY.54
89	MESSI	FF27EC	33	Male	Positive	Method 2	436237230	AY.44
90	MESSI	22D142	53	Male	Positive	Method 2	74000519	AY.118
91	MESSI	DE7C56	57	Male	Positive	Method 2	13924	AY.118
92	MESSI	D41350	44	Female	Positive	Method 2	100580235	AY.118
93	MESSI	0F6E02	36	Female	Positive	Method 2	273803376	B.1.2
94	MESSI	02608B	56	Male	Positive	Method 2	50325	BA.1.20
95	MESSI	F7190B	42	Female	Positive	Method 1	353829	BA.1.1.8
96	MESSI	2566E0	54	Female	Positive	Method 2	382134	BA.1.1.18
97	MESSI	8FDB69	42	Male	Positive	Method 1	110782171	BA.1.1
98	MESSI	3E7475	25	Male	Positive	Method 1	28583	BA.1.1
99	MESSI	FEC4E9	47	Male	Positive	Method 2	5284486	BA.1.1
100	MESSI	72BF40	53	Male	Positive	Method 1	3662	BA.1.1.8
101	MESSI	B5CC01	56	Female	Positive	N/A	N/A	BA.2.12.1
102	MESSI	ED6824	49	Female	Positive	N/A	N/A	BA.2.12.1
103	MESSI	ED6824	55	Male	Positive	Method 2	288002	BA.1.1
104	MESSI	B5CC01	48	Male	Positive	Method 2	2024081	BA.1.1
105	MESSI	150B4B	40	Male	Positive	Method 2	34165011	BA.1.1
106	MESSI	9C0AA4	34	Male	Positive	Method 1	87487	BA.1.1
107	MESSI	E674EB	49	Male	Positive	Method 1	9441043	BA.1.1
108	MESSI	12CE1E	51	Female	Positive	Method 1	7230964	BA.1.1

Viral RNA Extraction and Sequencing Library Preparation

[00105] Viral RNA was extracted from NP swabs in VTM using QIAamp Viral RNA Mini Kit (QIAGEN). Sequencing libraries were prepared using the Illumina COVIDSeq Test SARS-CoV-2 kit at a reduced quarter volume reaction on liquid handlers. Libraries were pooled at equal volume, and the pool's concentration and library size were quantified with the Invitrogen Qubit 4 Fluorometer and Agilent Tapestation. The final pool was sequenced on the Illumina NextSeq500 instrument using a 75 cycle High Output flow cell with 72 base pair single reads, 1.5pM loading concentration, and 5% PhiX v3 control spike-in.

Variant Analysis Pipeline

[00106] To classify COVID-19 variants, it is necessary to identify the mutations along each genome. To do this, a custom analytical pipeline was used based on the best practices workflow from GATK. The analysis starts with trimming Nextera adapters from each sequence, then individual reads with low quality scores (<q20) are eliminated. Next, the trimmed reads are aligned to the SARS-CoV-2 reference genome using BWA. In this study, the isolate Wuhan-Hu-1 obtained from GenBank (Accession Number: NC_045512.2) was used. Next, the customized GATK Workflow44 is automatically run to identify single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS) along the 29 kb of the SARS-CoV-2 genome. One pass of base quality score recalibration was used to generate high quality SNPs and INDELS, an important step for correcting errors produced during the alignment process and improves accuracy of variant calls. Next, a package called HaplotypeCaller was used to identify variants assuming a ploidy of "1". Generating a VCF file that undergoes hard filtering using the VariantFiltration command, and a summary statistics table that allows assessing the quality of each of the resulting genotypes. Finally, the VCF tables are used to generate consensus genomes in FASTA format using BCFtools v1.15.1.46 Next, each genome is concatenated into a larger file and processed with Pangolin v.4.1.2 to identify and classify the variant identity of each genome.

Quantitative RT-PCR

[00107] Method 1: SARS-CoV-2 PCR viral load – NP swab: Nasal swab VTM was aliquoted and cryopreserved from study subjects to determine SARS-CoV-2 N1 gene copy number by RT-PCR to stratify subjects as COVID PCR positive or negative. Viral RNA was extracted from 140 µL of VTM according to manufacturer's instructions (QiaAmp Viral RNA minikit). SARS-CoV-2 nucleocapsid (N1) and human RNase P (RPP30) RNA copies were determined using 5 µL of isolated RNA in the CDC-designed kit (CDC-006-00019, Revision: 03, Integrated DNA

Technologies 2019-nCoV kit). Standard quantitative RT-PCR (TaqPath 1-step RT qPCR Master Mix, Thermofisher) was run with NP1 RNA standard (Integrated DNA Technologies) and gene-specific standard curves (2e5 copy/mL – 20 copy/mL). Regression analysis was used to determine NP1 gene copy number and corrected to report copies/mL of VTM. Samples with a Ct value >35 are called as COVID PCR NEGATIVE and samples ≤35 are called COVID PCR POSITIVE.

[00108] Method 2: SARS-CoV-2 "High Sensitivity" qPCR viral load: Lab Developed Test (LDT) qPCR for SARS-CoV-2 Total RNA E-gene (envelope). Method: QIAGEN QIAsymphony DSP Virus/Pathogen Midi Kit (96)/QIAgility was used for isolation and purification of nucleic acids. PCR was run on Applied Biosystems QuantStudio 3 Real-Time PCR System. For each batch, a standard curve was run to extrapolate RNA copies/mL. NP/VTM (0.5ml) was diluted 1:1 with PBS and 0.8 mL input for RNA extractions (0.4 mL equivalent VTM). Assays were run in singlicate. Any positive VL detected is considered "Positive". Not detected is considered "Negative". The lower limit of quantification (LLOQ) for this assay was 128 RNA cp/mL for 0.4ml 1:1 diluted input. Values below the LOQ may be outside of the 95% confidence interval for reproducibility.

Microfluidic CoVariant-SPOT

[00109] **FIG. 5B** shows the microfluidic CoVariant-SPOT cassette. The cassette is based on designs as described in Heggestad, J. T. et al. Multiplexed, quantitative serological profiling of COVID-19 from blood by a point-of-care test. Sci Adv 7, (2021), which is incorporated by reference herein in its entirety, with changes including increasing the length of the vertical timing loops to compensate for the higher wettability of extraction/lysis buffers. In addition, the sample inlet was enlarged and moved to the top of the reaction chamber to allow for gravity driven sample introduction with a dropper. The reaction chamber was lengthened to prevent backflow into the wash reservoir. A delay channel was added between the reaction chamber and the wash reservoir to ensure that the sample completely settled into the reaction chamber prior to the release of wash buffer into the chamber.

[00110] The cassettes were fabricated using a laser ablation manufacturing process. Complementary layers of patterned acrylic (1mm Clarex, Astra Products) and double-sided adhesive (9474LE, 3M company) were fabricated using an LS900 Gravograph CO_2 laser cutter (Gravotech, Inc.) based on template files created in AutoCAD 2022 (Autodesk, Inc.). Layers were precisely assembled using a custom designed 3D-printed alignment device (**FIG. 11**). Final assembly included affixing the sample inlet and wash buffer reservoir to the top of the

cassette and adding the wicking pads to the outlet of the timing channel (Whatman CF7 100% cotton liner). The inlet reservoir and alignment device were designed in Solidworks 2019 and 3D printed using a Form 3 SLA printer (Form Labs, Inc.).

[00111] The cassette features an upper and a lower section of its reaction chamber with the same CoVariant-SPOT reagents as described before. Alexa Fluor 647 labeled anti-N antibody and anti-S antibody were mixed and deposited in the upper reaction chamber as three 100 nL spots at a concentration of 0.10 mg/mL for each antibody in 10% (w/v) trehalose 1x PBS. To aid in dissolution during testing, the detection antibodies were printed on top of three 10% (w/v) trehalose excipient pads in 1x PBS. In the lower chamber, one anti-N and three anti-S antibodies were inject printed in spatially distinct 360 pL spots diluted in 0.05% (w/v) trehalose in 1x PBS. To normalize for any gradient effect in the reaction chamber or for uneven excitation of the D4Scope laser, the four printed cAbs were randomly addressed in the reaction chamber. Additionally, four fiducial spots of anti-Cy5 antibody (Millipore Sigma, catalog number: C1117) at 0.33 mg/mL were printed adjacent to the cAbs. These spots were used for D4Scope alignment and as an assay control.

[00112] Analytical validation of the microfluidic CoVariant-SPOT was performed by testing a 6-point dose-response curve in triplicate with SARS-CoV-2 N and S1 proteins and an additional blank (n=4). As before, N and S1 proteins of the same SARS-CoV-2 variant were mixed and diluted with extraction buffer to a starting concentration of 300 ng/mL for each antigen, from which a series of 1/4th dilutions was obtained. To run the assay, 72 µL of sample was added to the sample inlet. Immediately after, 200 µL of wash buffer was added to the wash buffer inlet, and the device was left to incubate in the vertical position. Once the reaction chamber had completely drained of fluid, the cassette was inserted into the D4Scope, manually aligned using the fiducial spots, imaged, and analyzed. Microfluidic cassettes imaged on the D4Scope were analyzed in the same way as described in “CoVariant-SPOT testing procedure”. An objective outlier removal algorithm was used on the resulting calculated fluorescence intensities using Microsoft Excel. First, a minimum fluorescence threshold of 100 a.u. was assigned to any spot that fell below the threshold after background subtraction. Next, outliers were removed using two passes of a 1.5 times interquartile range removal criteria. If greater than 50% of capture spots for a single cAb on a cassette were flagged as outliers, they would be removed from analysis (this did not occur in this study).

D4Scope

[00113] The D4Scope has been described in greater detail in Fontes, C. M. et al. Ultrasensitive point-of-care immunoassay for secreted glycoprotein detects Ebola infection earlier than PCR. Sci Transl Med 13, (2021) and Heggestad et al., 2021 (referenced earlier), both of which are incorporated by reference herein in their entirety. A modification made to the D4Scope for this study was the introduction of a higher power excitation laser with a higher quality collimator lens. Briefly, the D4Scope is composed of a Basler Ace CMOS Camera module (AcA3088-57um, Basler AG), 676/37-25 nm bandpass filter (Semrock), MC100X lens (Optoengineering), and an obliquely angled (30°) 638nm red laser module (Sharp). Mounts and housings for the optical components and holder for the microfluidic cassette holder were 3D printed via selective laser sintering with a Formlabs Fuse 1 (Formlabs Inc.). The D4Scope can be controlled either from a built-in Raspberry Pi 4B 2GB (Raspberry Pi Foundation) with accompanying 3.5" TFT LCD display (UCTRONCIS) or directly from a personal computer via a USB 3.0 connection.

Results

Fabrication of CoVariant-SPOT

[00114] CoVariant-SPOT is a multiplexed sandwich immunoassay where all the biomolecular reagents needed to complete the assay are stored stably on a poly(oligoethylene glycol methyl ether methacrylate) (POEGMA) surface coating (**FIG. 1** (A)) as described in Heggestad, J. T., Fontes, C. M., Joh, D. Y., Hucknall, A. M. & Chilkoti, A. In Pursuit of Zero 2.0: Recent Developments in Nonfouling Polymer Brushes for Immunoassays. Adv Mater 32, e1903285, doi:10.1002/adma.201903285 (2020) and Hucknall, A. et al. Simple Fabrication of Antibody Microarrays on Nonfouling Polymer Brushes with Femtomolar Sensitivity for Protein Analytes in Serum and Blood. Advanced Materials 21, 1968-1971, (2009), both of which are incorporated by reference herein in their entirety. Multiplexing is accomplished using inkjet printing of spatially discrete immobilized capture antibodies (cAbs) that exhibit differential levels of binding to the S proteins of each variant. Nearby, a fluorescently labeled detection antibody (dAb) which binds similarly to the S proteins of all variants is co-printed with an excipient (trehalose), making the dAbs "dissolvable" upon addition of biological fluid. In addition, an antibody pair for N was incorporated into the assay, as it is expressed more abundantly than S and is more conserved across SARS-CoV-2 variants. After incubation with a sample, variation in the fluorescence intensity at the cAb spots allows diagnosis of COVID-19 infection (based on N) and identification of the likely SARS-CoV-2 variant causing infection (**FIG. 1** (B)).

[00115] To identify antibodies for CoVariant-SPOT, high throughput screens were conducted to determine optimal cAb/dAb pairs that bound to SARS-CoV-2 variants differentially. For antibodies targeting S, 29 potential cAbs and 13 potential dAbs were screened against WT, Delta, and Omicron S1 proteins, resulting in 1131 different dose-response curves. The antibodies against Beta S1 were also screened, leading to a total of 1508 dose-response curves. All antibodies tested are listed in **Table 2**. In this screening process, all 29 candidate cAbs are inkjet printed onto POEGMA coated slides in a microarray. Next, recombinant S1 proteins for each variant are individually spiked into fetal bovine serum at multiple concentrations and added to the arrays containing all 29 cAbs. After a 30-minute incubation, slides are washed, and then a dAb is added to complete the sandwich formation process, resulting in 29 dose-response curves per dAb per S1 protein. After repeating this process for each dAb and S1 protein variant, three potential cAbs were identified for S1 (for a given dAb) that could potentially be used to differentiate between WT, Delta, and Omicron, depending on the fluorescence output at each cAb spot. The anti-S1 antibody pairs identified also bind to the S trimers for WT, Delta, and Omicron variants similarly compared to S1 (**FIG. 6A and FIG. 6B**). Therefore, the final version of the CoVariant-SPOT featured four cAbs (three targeting S and one targeting N) and a dAb cocktail including of one dAb targeting S and one dAb targeting N. Of note, these high throughput antibody screens can be performed rapidly—on the order of a couple of days—which enables rapid incorporation of more antibodies into CoVariant-SPOT as new variants emerge or to better discriminate between variants.

Analytical performance of CoVariant-SPOT

[00116] To investigate the analytical performance of CoVariant-SPOT, the response to WT, Delta, and Omicron recombinant S1 and N proteins were evaluated at various dilutions. Experiments were performed in a commercially available extraction/lysis buffer (Acro Biosystems). To build dose-response curves, S1 and N proteins for WT, Delta, and Omicron were added to CoVariant-SPOT at 14 dilutions starting at 300 ng/mL as the highest concentration. The results are shown in **FIG. 2A**. For this experiment, a 1 h incubation was used; however, in a separate set of experiments it was found the time can be lowered to 15 min with only a modest impact on analytical sensitivity (**FIG. 7**). Dose-response curves were fit using a 5-parameter logistic regression and the limit-of-detection (LOD) was calculated, as described elsewhere. A summary of the LODs is shown in **FIG. 2B**.

Table 2. Antibodies tested in the S high-throughput screen which includes 29 anti-S antibodies. Antibodies in bold were included in CoVariant-SPOT.

Antibody ID	Publication Name	Supplier
1	DH1041	DHVI
2	DH1042	DHVI
3	DH1043	DHVI
4	DH1111	DHVI
5	DH1284	DHVI
6	DH1193	DHVI
7	DH1044	DHVI
8	DH1047	DHVI
9	DH1049	DHVI
10	DH1050.1	DHVI
11	DH1051	DHVI
12	DH0148	DHVI
13	DH1054	DHVI
14	DH1053	DHVI
15	DH1055	DHVI
16	LT8010	Leinco Technologies
17	LT5000	Leinco Technologies
18	LT4000	Leinco Technologies
19	**S1N-M122**	**Acro Biosystems**
20	**S1N-M130**	**Acro Biosystems**
21	40150-D001	Sino Biological
22	40150-D002	Sino Biological
23	40150-D003	Sino Biological
24	40150-D004	Sino Biological
25	**40591-MM43**	**Sino Biological**
26	**40591-MM48**	**Sino Biological**
27	40592-R001	Sino Biological
28	40592-R117	Sino Biological
29	40592-R118	Sino Biological

[00117] Importantly, WT, Delta, and Omicron can be differentiated by the relative fluorescence of the anti-S cAb spots, likely due to some cAbs having weaker binding affinities to VOC S1 proteins because of mutations relative to WT. **FIG. 2C** shows the ratio of MM43/MM48 plotted

against MM48/AM130 (left) and MM43/AM130 (right). On both plots, Delta can be clearly differentiated from WT and Omicron across S1 concentrations ranging from 100 ng/mL to 0.4 ng/mL if MM43/MM48 is greater than ~1.0, and/or MM48/AM130 is less than ~1.0. Conversely, Omicron and WT can only be differentiated at higher concentrations of Omicron S1 if MM43/MM48 is less than ~0.75 or MM43/AM130 is less than ~0.8, suggesting that MM43 does not bind as well to Omicron S1. The MM43 cAb also binds poorly to the BA.2 sub lineage of Omicron (**FIG. 8**). Of note, antibodies that bind to WT but do not bind—or bind weakly—to S proteins from certain variants has been observed with other antibodies and is potentially one of the drivers of SARS-CoV-2 variant escape from natural or vaccine induced humoral immunity.

[00118] Next, the performance of CoVariant-SPOT was investigated to detect SARS-CoV-2 virus samples that had been propagated in cultured cells and inactivated using ultraviolet (UV) irradiation. Samples for WT, Delta, and Omicron viruses were added to lysis buffer, and then added to CoVariant-SPOT assays at various dilutions. The resulting dose-response curves are shown in **FIG. 3**, which plot the normalized intensity against median tissue culture infectious dose per milliliter ($TCID_{50}$/mL). The analytical sensitivity in terms of $TCID_{50}$/mL for N is superior to that of S1, likely because N is expressed more abundantly than S. Similar trends in terms of cAb specificity for each variant were also observed. Notably, MM48 cAb does not bind as efficiently to Delta S and MM43 cAb does not bind efficiently to Omicron S. By examining the ratio of different anti-S cAb, similar patterns exist compared to the recombinant samples (**FIG. 9**), further supporting the hypothesis that CoVariant-SPOT can differentiate between variants, especially at high viral loads. In a separate experiment, it was found that the Acro Biosystems lysis/extraction buffer performed better in terms of analytical sensitivity compared to standard viral transport media (VTM) (**FIG. 10**). Overall, these experiments strongly suggest that CoVariant-SPOT could be useful to diagnose COVID-19 and differentiate between specific SARS-CoV-2 variants based on the fluorescence output of the assay.

Assessment of CoVariant-SPOT against clinical specimens

[00119] As proof-of-principle, CoVariant-SPOT was used to diagnose COVID-19 infection and differentiate between Delta and Omicron variants in clinical specimens. To demonstrate the clinical performance of CoVariant-SPOT, biobanked NP swab samples from 32 COVID-19 negative and 76 positive individuals were tested (**Table 1**). All samples were collected in VTM or universal transport media (UTM) and confirmed as COVID-19 positive or negative via reverse transcriptase polymerase chain reaction (RT-PCR). For a subset of the samples, viral load was quantified using quantitative RT-PCR. Of the 76 COVID-19 samples, 62 were sequenced using

Illumina NextSeq500. Although the remaining 14 positive samples were not sequenced due to sample volume limitations, the probability of infection being from the predicted variant is high based on surveillance data collected by GISAID. An unavoidable limitation of this study is the use of biobanked samples as: (1) samples were collected in VTM/UTM rather than a lysis buffer that helps extract N and S proteins, (2) the samples were collected in a large volume (~3 mL) which causes significant protein dilution relative to ideal collection methods (~150 µL), and (3) the samples were stored at -80 °C rather than tested fresh.

[00120] For clinical validation, each sample was tested in duplicate on CoVariant-SPOT. The ability of CoVariant-SPOT to diagnose COVID-19 was first examined via detection of N protein. The aggregate data for all samples is shown in **FIG. 4A**. It was found that a statistically significant difference between the mean intensity for COVID-19–positive and –negative samples ($P < 0.001$), as determined by a two-tailed unpaired t-test. Sensitivity and specificity were determined by receiver operator curve (ROC) analysis (**FIG. 4B**) which yielded an area under the ROC curve (AUC) of 0.87. At the optimal cut point of 2.72 arbitrary units for N, the sensitivity is 68.4% (95% CI: 57.3% – 77.8%) and the specificity is 96.9% (95% CI: 84.3% – 99.8%). For a subset of the positive samples, viral load was quantified using quantitative RT-PCR. It was found that the D4 intensity for the N antibody pair was highly correlated with viral load (R^2 = 0.72) (**FIG. 4C**).

[00121] Next, the ability of CoVariant-SPOT to differentiate between two VOCs—Delta and Omicron—was examined via detection of S protein. To test how well the two VOCs can be discriminated, MM43/MM48 was plotted against MM48/AM130 and MM43/AM130 for all samples where the 1B2—the cAb for N—intensity is greater than 2.72 arbitrary units (i.e., tested positive), as shown in **FIG. 4D**. It was found that Delta is readily discriminated from Omicron if MM43/MM48 is greater than 0.99. Consistent with results from recombinant samples and UV inactivated viruses, discrimination between Delta and Omicron improves with increasing viral load, as shown in **FIG. 4E.** These results suggest that the discriminatory power of CoVariant-SPOT is improved for samples with high viral load and that the overall performance would likely improve in a prospective study where samples are collected in a small volume with extraction buffer.

Integration into a point-of-care format

[00122] Finally, it was sought to demonstrate the deployability of the test at the POC, de-coupled from laboratory or clinical infrastructure. To do this, CoVariant-SPOT was integrated into a microfluidic cassette. The microfluidic cassette automates CoVariant-SPOT passively with

capillary and gravity driven flow and requires users to only add sample and wash buffer at the time of testing. Further, the microfluidic cassettes can be imaged with a portable, low-cost, and easy-to-use fluorescent detector—the D4Scope. The D4Scope costs ~$1,000 and can be operated using either battery or wall power. Combined, the microfluidic cassette and D4Scope could allow for sample testing and variant discrimination to occur simply by swabbing the nose, adding the swab to extraction buffer, adding a few drops to the microfluidic cassette, and imaging on the D4Scope after an incubation period (**FIG. 5A**).

[00123] For this study, a modified version of the microfluidic cassette was used that enables testing from viral extraction buffer and improves the user-friendliness of device operation. Specifically, the timing channel was lengthened to account for the increased wettability of the extraction buffer compared to serum/plasma or blood—the sample types that the D4 microfluidic cassette was originally designed for—and the inlet and reaction chamber were modified to make sample introduction significantly easier. This inlet allows the user to load the sample with a dropper rather than a micropipette (**FIG. 5B, FIG. 11**). To perform a test, ~72 µL of sample is added by dropper to the sample inlet, followed by addition of wash buffer to the second well. All assay reagents are inkjet printed within the reaction chamber. After sample addition, the four steps of the D4 assay take place in the reaction chamber, resulting in the formation of antibody sandwiches with the analyte. Simultaneously, a small volume of sample traverses the snaking timing channels, which govern the incubation time. Once the sample reaches the outlet of the timing channel, the sample is absorbed into a wicking pad that is situated at the outlet of the timing channel. This removes sample and unbound reagent from the reaction chamber, while also flushing the chamber with wash buffer. Once the wash buffer is also absorbed, a clean and dry surface is ready for imaging on the D4Scope. To validate the performance of the microfluidic CoVariant-SPOT, WT, Delta, and Omicron recombinant S1 and N proteins were tested at various dilutions. The resulting dose-response curves are shown in **FIG. 5C**. Similar to the results presented in **FIG. 2**, the intensity at MM48 cAb spots is attenuated for Delta S1 and the intensity of MM43 cAb is attenuated for Omicron S1, suggesting that the microfluidic CoVariant-SPOT can distinguish between Delta and Omicron. As a proof-of-principle, a subset of clinical samples were tested using the microfluidic CoVariant-SPOT and it was found that it could reliably distinguish between Delta and Omicron by examining the anti-S cAb ratios (**FIG. 5D**).

[00124] As the COVID-19 pandemic has progressed, epidemiological surveillance of emerging SARS-CoV-2 VOCs has proven important in guiding the public health response. Currently, SARS-CoV-2 VOCs are identified by NGS following a positive diagnosis and surveilled using sequence repositories like GISAID, while viral RNA detection by RT-PCR

remains the gold standard for COVID-19 diagnosis. Both NGS and RT-PCR, however, are resource-intensive processes that rely on centralized testing models, drastically limiting their use in LMICs. The inevitable result of this disparity is an incomplete global epidemiological understanding of the COVID-19 pandemic. Despite their comparatively lower sensitivity, antigen detection via LFAs has proven to be a useful supplement to RT-PCR diagnosis due to their rapid time-to-result, ease-of-use, and low cost. While rapid antigen tests represent the most promising solution to global disparities in access to COVID-19 testing, currently available tests are unable to differentiate between SARS-CoV-2 VOCs.

[00125] The disclosed assay can address this need by enabling simultaneous diagnosis of COVID-19 and differentiation between SARS-CoV-2 strains in an easy-to-use POC platform. Although the platform can be easily expanded to detect other variants, as proof-of-principle, it was chosen to limit the target strains in the validation studies to the WT, Delta, and Omicron variants. A major strength of the disclosed assay platform is the highly multiplexed nature of the microarray format that has two useful attributes for VOC identification. First, the microarray format of CoVariant-SPOT can also be used as a high-throughput antibody screening platform. To identify antibodies useful for VOC identification by CoVariant-SPOT, a panel of 29 commercially available and in-house antibodies as cAb's were printed in a D4 microarray and compared the binding of each antibody to VOC specific antigens, that enabled the identification of a subset of antibodies with differing sensitivity against WT, Delta, and Omicron. This approach will continue to be useful to enable differentiation of new VOCs as they emerge. Second, the microarray format of CoV-SPOT enables discrimination between VOC's.

[00126] In this study, the performance of CoVariant-SPOT was validated with recombinant antigens, UV inactivated virus, and clinical samples. Testing with recombinant antigens confirmed that the selected antibodies can reliably distinguish between WT, Delta, and Omicron via their differential binding to the S1 proteins. In addition, CoVariant-SPOT includes an antibody pair for N that was similarly sensitive to all recombinant N protein variants tested, despite four mutations in Delta and six in Omicron N relative to WT. These results were recapitulated in UV-inactivated SARS-CoV-2 virus isolates, further supporting the ability to differentiate variants using CoVariant-SPOT. In a proof of principle study with clinical samples, it was confirmed that N is the more useful diagnostic target compared to S, and that it could differentiate samples collected during the Delta wave in the US from those collected during the Omicron wave by the difference on the fluorescence intensity from the printed spots of different anti-S specific cAb's. It was also shown that CoVariant-SPOT can be implemented in an easy-

to-use microfluidic cassette, potentially enabling testing to occur anywhere, decoupled from clinical infrastructure.

[00127] SARS-CoV-2 variant surveillance in the US (and worldwide) has been lackluster due to the difficulty in implementing NGS within the current clinical workflow. This is also true in many LMICs where access to facilities that can conduct NGS is cost prohibitive or non-existent. CoVariant-SPOT can be used as a valuable adjunct to NGS that could enhance surveillance capabilities in LMICs. While NGS will remain a useful tool for identifying new variants, recognizing recurring SNPs, and tracking sequence evolution, the disclosed assay is far better equipped to readily determine strain dominance down to the community level, regardless of available resources. Moreover, identifying COVID-19 strain can help personalize treatment. For instance, strain identification is clinically relevant because currently available therapeutic monoclonal antibodies have less therapeutic efficacy against certain variants (e.g., Omicron).

[00128] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention.

[00129] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the devices, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

[00130] For reasons of completeness, various aspects of the invention are set out in the following numbered clauses:

[00131] Clause 1. A method of detecting different SARS-CoV-2 variants, the method comprising: contacting a biological sample with a device, the device comprising

a substrate,

a non-fouling layer positioned on the substrate, the non-fouling layer comprising a brush-like polymer,

a capture region positioned on the non-fouling layer, the capture region comprising an array of capture agents, each individual capture agent located at a discrete location on the non-fouling layer, wherein each individual capture agent is capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array, and

a detection region positioned on the non-fouling layer spatially separated from the capture region, the detection region comprising at least one detection agent and an excipient, wherein the at least one detection agent solubilizes upon contacting the biological sample and is capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins; and

detecting the presence or absence of different SARS-CoV-2 variants in the biological sample, wherein the presence or absence of different SARS-CoV-2 variants is detected by measuring a detectable signal provided by each complex formed at each discrete location.

[00132] Clause 2. The method of clause 1, wherein the capture region comprises an array of threshold capture agents, each individual threshold capture agent located at a discrete location on the non-fouling layer, wherein each individual threshold capture agent is capable of specifically binding to one or more nucleocapsid proteins of one or more SARS-CoV-2 variants, and the detection region comprises at least one detection agent capable of specifically binding to a complex formed between an individual threshold capture agent and the one or more nucleocapsid proteins.

[00133] Clause 3. The method of clause 1 or 2, wherein the array of capture agents comprises 2 to 30 capture agents, each individual capture agent capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array.

[00134] Clause 4. The method of clause 2, wherein the array of threshold capture agents comprises 1 to 10 threshold capture agents, each individual threshold capture agent capable of specifically binding to one or more nucleocapsid proteins of one or more SARS-CoV-2 variants.

[00135] Clause 5. The method of any one of clauses 2-4, wherein the detection region comprises 1 to 5 detection agents, each individual detection agent capable of specifically binding to at least one complex formed between an individual capture agent and the one or more spike proteins or capable of specifically binding to at least one complex formed between an individual threshold capture agent and the one or more nucleocapsid proteins.

[00136] Clause 6. The method of any one of clauses 1-5, comprising a ratio of total capture agent to total detection agent of 2:1 to 5:1 (total capture agent:total detection agent).

[00137] Clause 7. The method of any one of clauses 2-6, wherein the capture region comprises three individual capture agents and one individual threshold capture agent, and the detection region comprises one individual detection agent capable of specifically binding to a

complex formed between an individual capture agent and the one or more spike proteins, and an individual detection agent capable of specifically binding to a complex formed between the threshold capture agent and the one or more nucleocapsid proteins.

[00138] Clause 8. The method of any one of clauses 1-7, wherein each capture agent and each threshold capture agent, individually, comprises a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, a nucleic acid, or a combination thereof.

[00139] Clause 9. The method of any one of clauses 1-8, wherein each capture agent and each threshold capture agent, individually, comprises an antibody or a fragment thereof.

[00140] Clause 10. The method of any one of clauses 1-9, wherein the at least one detection agent comprises a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, or a combination thereof.

[00141] Clause 11. The method of any one of clauses 1-10, wherein the at least one detection agent comprises an antibody or fragment thereof.

[00142] Clause 12. The method of any one of clauses 1-11, wherein the at least one detection agent comprises a detectable signal selected from the group consisting of a colorimetric signal, a fluorescent signal, a radioactive signal, a magnetic signal, and an enzymatic signal.

[00143] Clause 13. The method of any one of clauses 1-12, wherein the excipient comprises a salt, a carbohydrate, a polyol, an emulsifier, a water soluble polymer, or a combination thereof.

[00144] Clause 14. The method of any one of clauses 1-13, wherein the excipient comprises trehalose.

[00145] Clause 15. The method of any one of clauses 1-14, wherein the brush-like polymer comprises a monomer core group and a protein-resistant head group coupled to the monomer core group.

[00146] Clause 16. The method of any one of clauses 1-15, wherein the brush-like polymer comprises poly(oligo(ethylene glycol)methyl methacrylate) (POEGMA).

[00147] Clause 17. The method of any one of clauses 1-16, wherein the sample comprises a nasal or nasopharyngeal swab, a throat swab, or saliva.

[00148] Clause 18. The method of any one of clauses 1-17, wherein the substrate comprises a glass, a silicon, a metal oxide, a polymer, or a combination thereof.

[00149] Clause 19. The method of any one of clauses 1-18, wherein the method is capable of simultaneously detecting the presence or absence of at least two different SARS-CoV-2 variants in the biological sample.

[00150] Clause 20. The method of any one of clauses 1-19, wherein detecting the presence or absence of different SARS-CoV-2 variants occurs in less than or equal to 60 minutes after the biological sample contacts the device.

[00151] Clause 21. The method of any one of clauses 1-20, wherein each SARS-CoV-2 variant has at least one mutation of one or more spike proteins that is different from the other SARS-CoV-2 variants.

[00152] Clause 22. The method of any one of clauses 1-21, wherein the method has a limit of detection (LOD) for nucleocapsid of less than or equal 0.07 ng/mL.

[00153] Clause 23. The method of any one of clauses 1-22, wherein a subject receives a treatment dependent on the presence or the absence of different SARS-CoV-2 variants.

[00154] Clause 24. The method of any one of clauses 1-23, wherein measuring the detectable signal is done visually or with a smart device.

CLAIMS

What is claimed is:

1. A method of detecting different SARS-CoV-2 variants, the method comprising:

contacting a biological sample with a device, the device comprising

a substrate,

a non-fouling layer positioned on the substrate, the non-fouling layer comprising a brush-like polymer,

a capture region positioned on the non-fouling layer, the capture region comprising an array of capture agents, each individual capture agent located at a discrete location on the non-fouling layer, wherein each individual capture agent is capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array, and

a detection region positioned on the non-fouling layer spatially separated from the capture region, the detection region comprising at least one detection agent and an excipient, wherein the at least one detection agent solubilizes upon contacting the biological sample and is capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins; and

detecting the presence or absence of different SARS-CoV-2 variants in the biological sample, wherein the presence or absence of different SARS-CoV-2 variants is detected by measuring a detectable signal provided by each complex formed at each discrete location.

2. The method of claim 1, wherein the capture region comprises an array of threshold capture agents, each individual threshold capture agent located at a discrete location on the non-fouling layer, wherein each individual threshold capture agent is capable of specifically binding to one or more nucleocapsid proteins of one or more SARS-CoV-2 variants, and

the detection region comprises at least one detection agent capable of specifically binding to a complex formed between an individual threshold capture agent and the one or more nucleocapsid proteins.

3. The method of claim 1, wherein the array of capture agents comprises 2 to 30 capture agents, each individual capture agent capable of specifically binding to one or more spike proteins of one or more SARS-CoV-2 variants with a different affinity than the other capture agents of the array.

4. The method of claim 2, wherein the array of threshold capture agents comprises 1 to 10 threshold capture agents, each individual threshold capture agent capable of specifically binding to one or more nucleocapsid proteins of one or more SARS-CoV-2 variants.

5. The method of claim 2, wherein the detection region comprises 1 to 5 detection agents, each individual detection agent capable of specifically binding to at least one complex formed between an individual capture agent and the one or more spike proteins or capable of specifically binding to at least one complex formed between an individual threshold capture agent and the one or more nucleocapsid proteins.

6. The method of claim 1, comprising a ratio of total capture agent to total detection agent of 2:1 to 5:1 (total capture agent:total detection agent).

7. The method of claim 2, wherein the capture region comprises three individual capture agents and one individual threshold capture agent, and

the detection region comprises one individual detection agent capable of specifically binding to a complex formed between an individual capture agent and the one or more spike proteins, and one individual detection agent capable of specifically binding to a complex formed between the threshold capture agent and the one or more nucleocapsid proteins.

8. The method of claim 1, wherein each individual capture agent comprises a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, a nucleic acid, or a combination thereof.

9. The method of claim 1, wherein each individual capture agent comprises an antibody or a fragment thereof.

10. The method of claim 1, wherein the at least one detection agent comprises a peptide, a protein, a carbohydrate, a lipid, a small molecule ligand, or a combination thereof.

11. The method of claim 1, wherein the at least one detection agent comprises an antibody or fragment thereof.

12. The method of claim 1, wherein the at least one detection agent comprises a detectable signal selected from the group consisting of a colorimetric signal, a fluorescent signal, a radioactive signal, a magnetic signal, and an enzymatic signal.

13. The method of claim 1, wherein the excipient comprises a salt, a carbohydrate, a polyol, an emulsifier, a water soluble polymer, or a combination thereof.

14. The method of claim 1, wherein the excipient comprises trehalose.

15. The method of claim 1, wherein the brush-like polymer comprises a monomer core group and a protein-resistant head group coupled to the monomer core group.

16. The method of claim 1, wherein the brush-like polymer comprises poly(oligo(ethylene glycol)methyl methacrylate) (POEGMA).

17. The method of claim 1, wherein the sample comprises a nasal or nasopharyngeal swab, a throat swab, or saliva.

18. The method of claim 1, wherein the substrate comprises a glass, a silicon, a metal oxide, a polymer, or a combination thereof.

19. The method of claim 1, wherein the method is capable of simultaneously detecting the presence or absence of at least two different SARS-CoV-2 variants in the biological sample.

20. The method of claim 1, wherein detecting the presence or absence of different SARS-CoV-2 variants occurs in less than or equal to 60 minutes after the biological sample contacts the device.

21. The method of claim 1, wherein each SARS-CoV-2 variant has at least one mutation of one or more spike proteins that is different from the other SARS-CoV-2 variants.

22. The method of claim 1, wherein the method has a limit of detection (LOD) for nucleocapsid of less than or equal 0.07 ng/mL.

23. The method of claim 1, wherein a subject receives a treatment dependent on the presence or the absence of different SARS-CoV-2 variants.

24. The method of claim 1, wherein measuring the detectable signal is done visually or with a smart device.

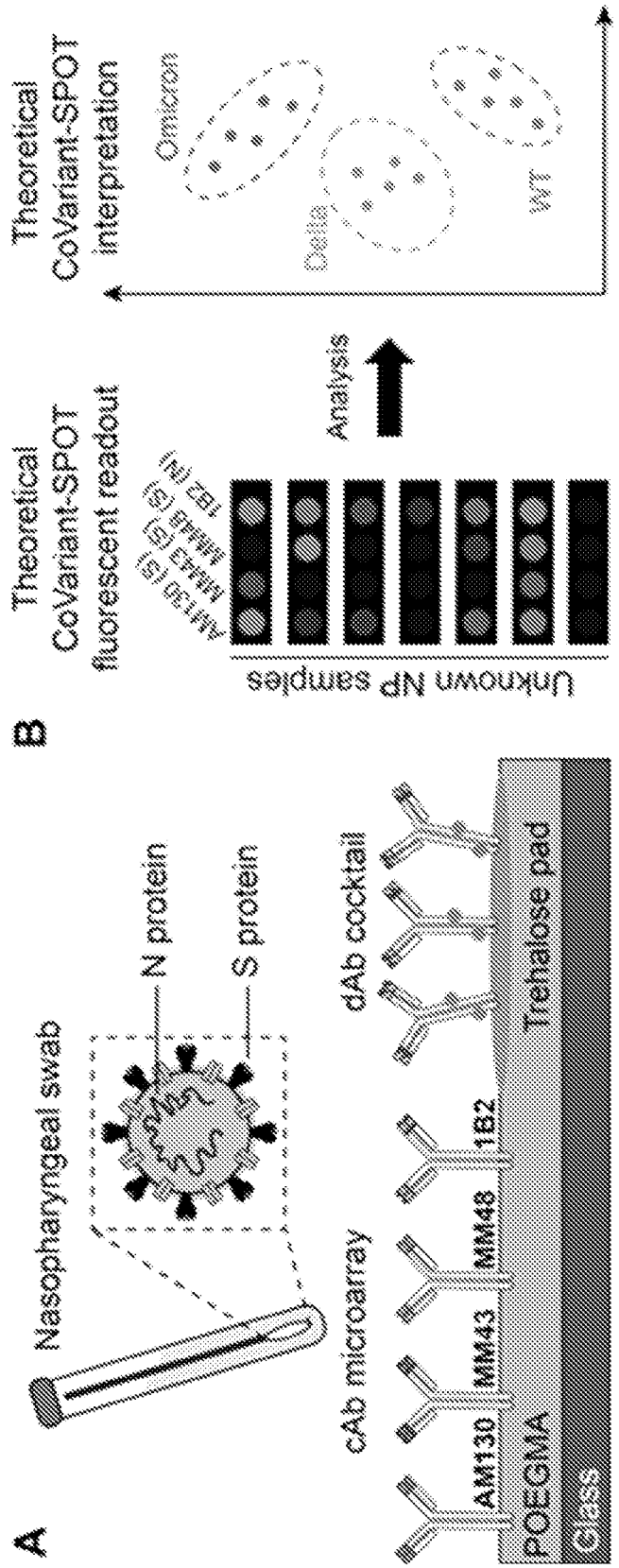
A
Nasopharyngeal swab
N protein
S protein
dAb cocktail
cAb microarray
AM130
MM43
MM48
1B2
POEGMA
Trehalose pad
Glass
B
Unknown NP samples
AM130 (1)
MM43 (2)
MM48 (3)
1B2 (4)
Theoretical CoVariant-SPOT fluorescent readout
Analysis
Theoretical CoVariant-SPOT interpretation
Omicron
Delta
WT

FIG. 1

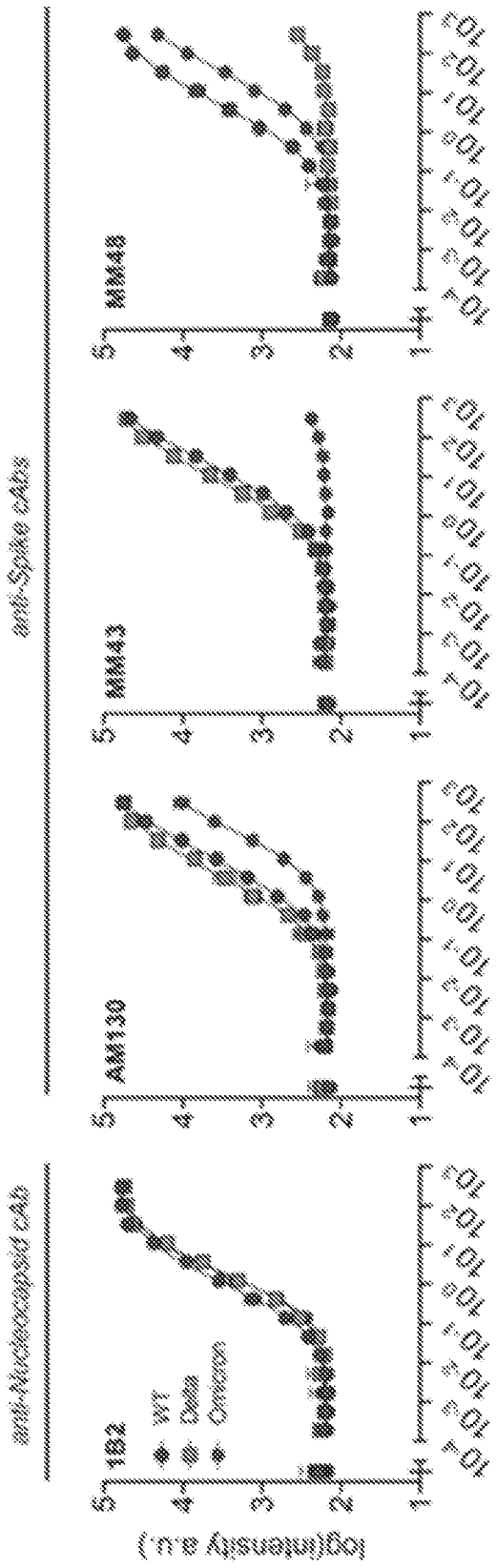
anti-Nucleocapsid cAb
anti-Spike cAbs
162
AM130
MM43
MM46
WT
Delta
Omicron
log(intensity a.u.)

FIG. 2A

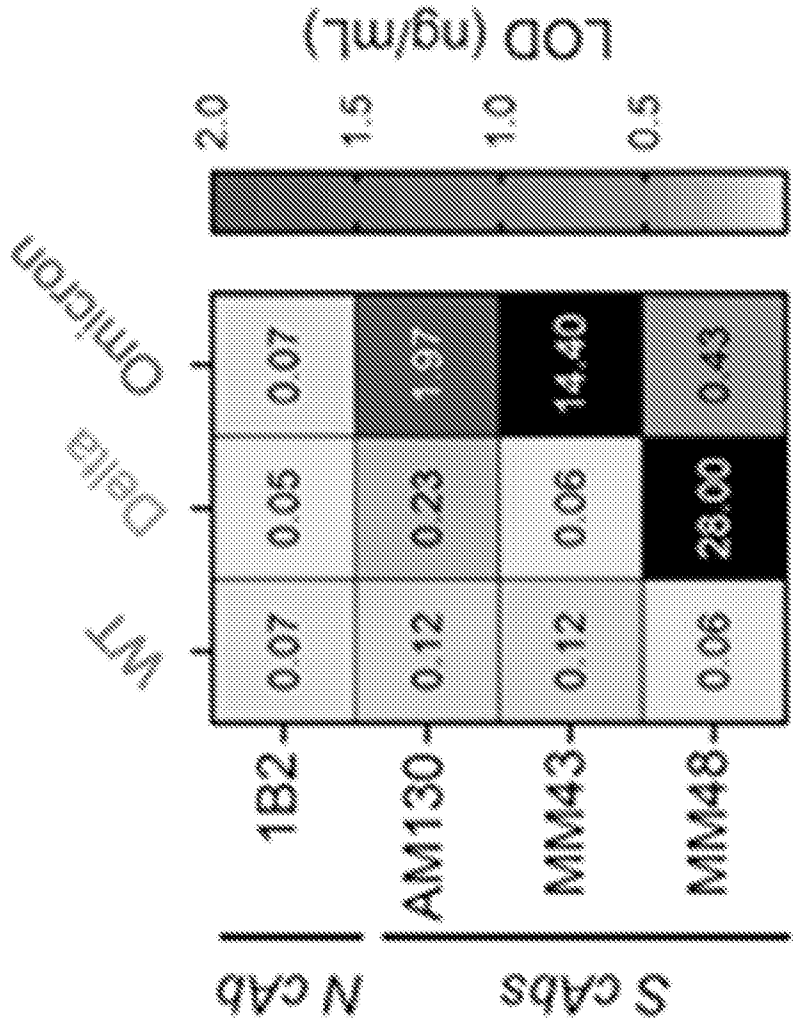
LOD (ng/mL)
2.0
1.5
1.0
0.5
WT
Delta
Omicron
0.07
0.05
0.07
1.67
0.23
0.12
14.40
0.06
0.12
0.43
28.03
0.06
1B2
AM130
MM43
MM48
N cAb
S cAbs

FIG. 2B

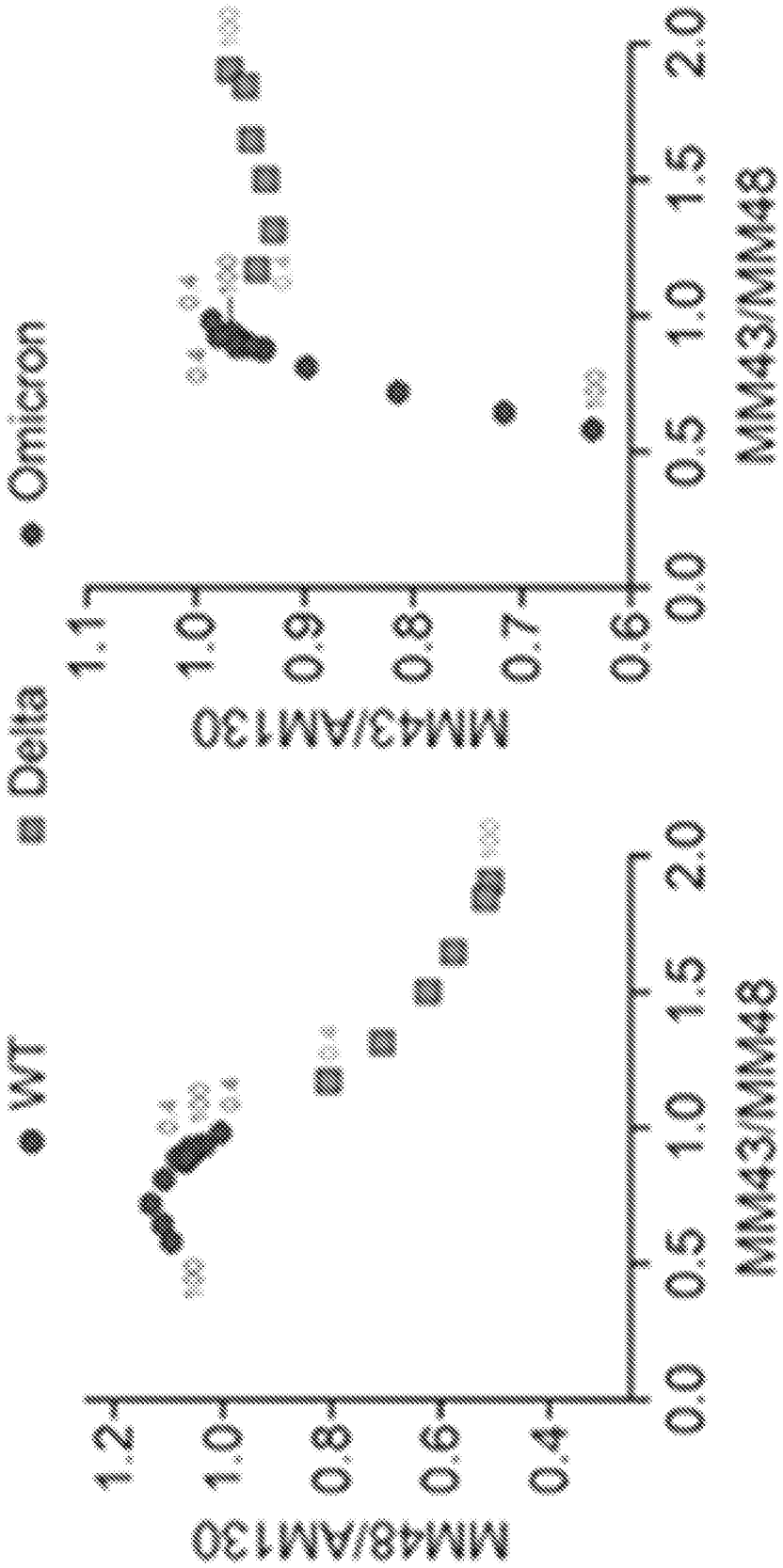

FIG. 2C

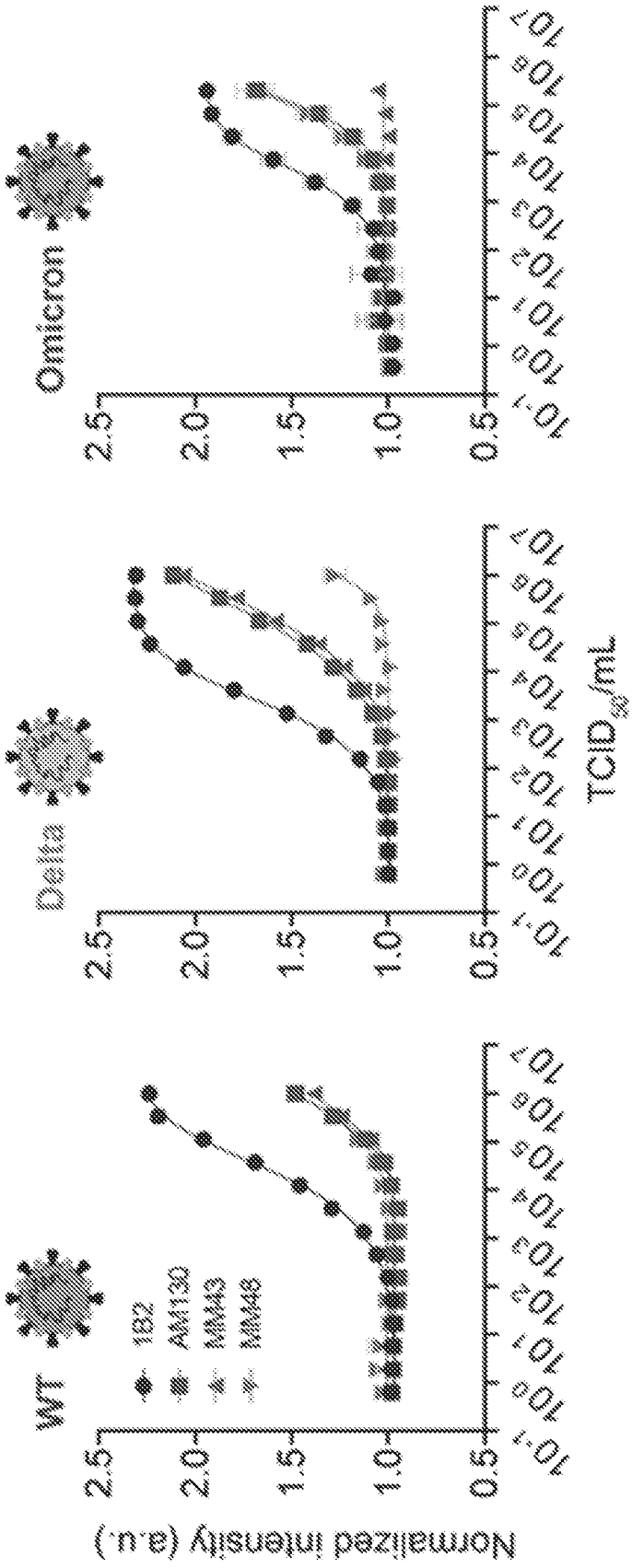
WT
Delta
Omicron
182
AM130
MM43
MM48
Normalized intensity (a.u.)
TCID50/mL
2.5
2.0
1.5
1.0
0.5
10-1
100
101
102
103
104
105
106
107

FIG. 3

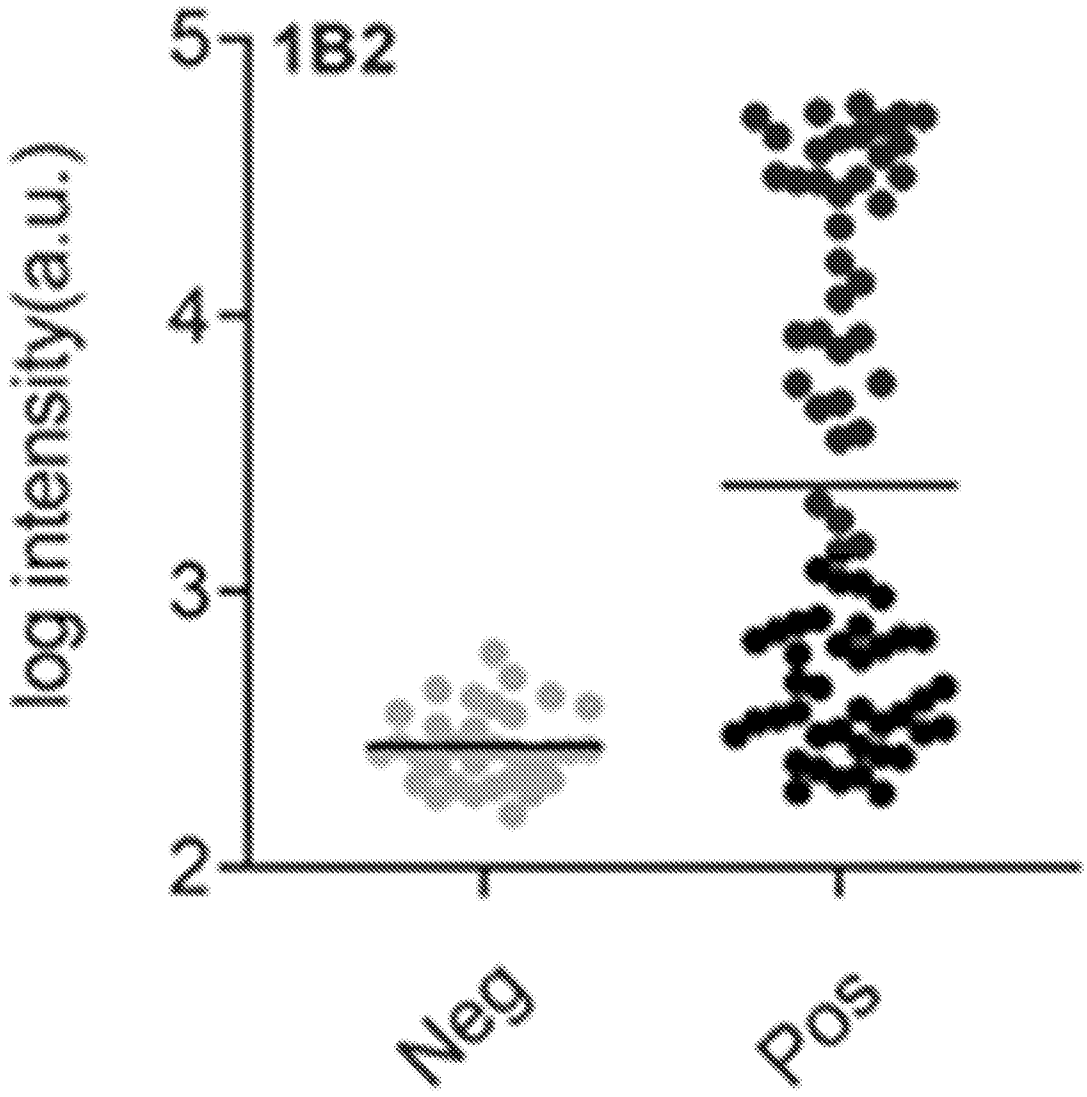

FIG. 4A

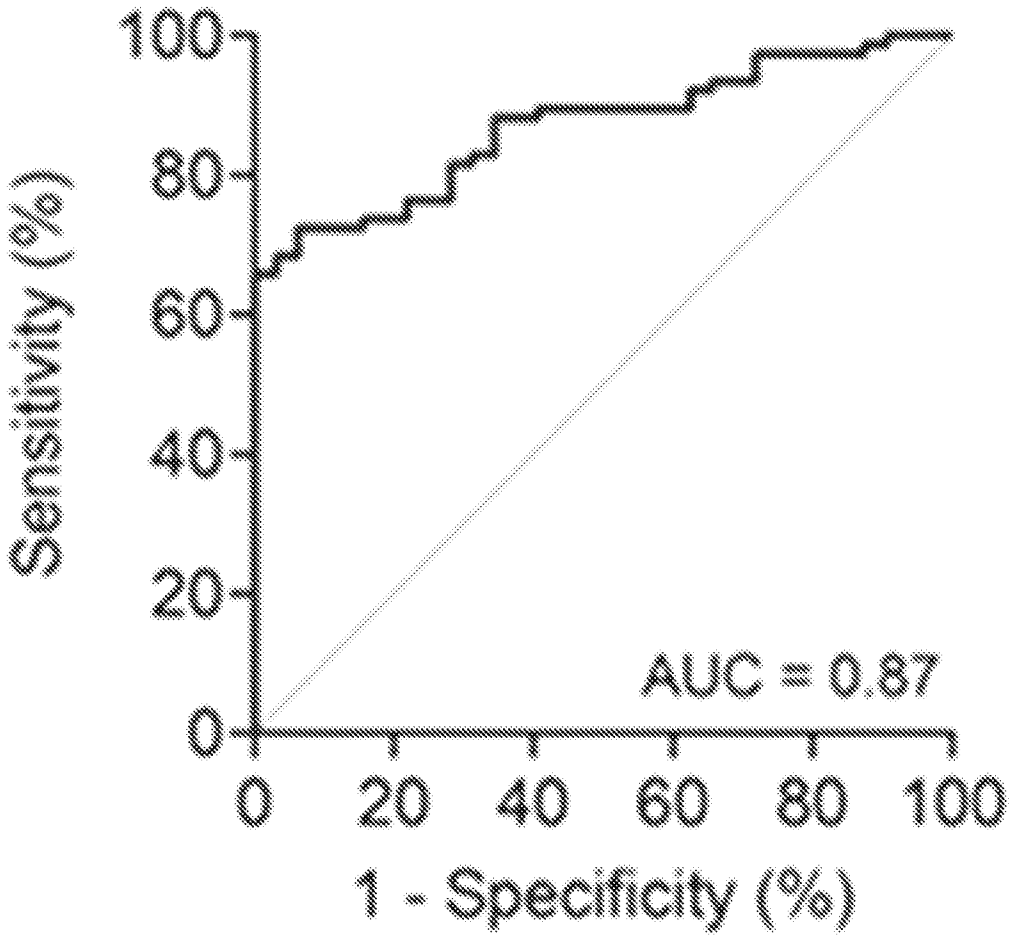
100
80
60
40
20
0
Sensitivity (%)
AUC = 0.87
0
20
40
60
80
100
1 - Specificity (%)

FIG. 4B

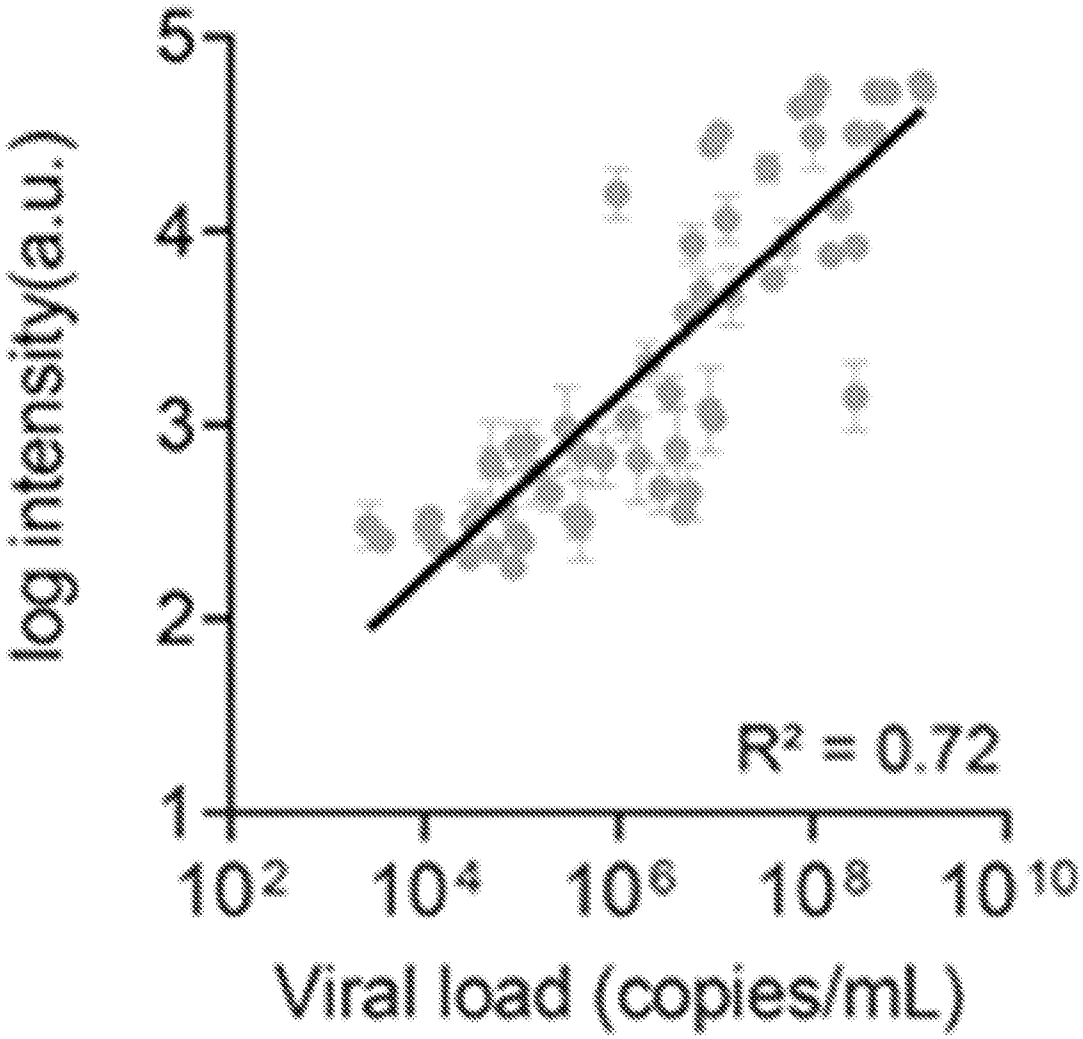
log intensity(a.u.)
5
4
3
2
1
$R^2 = 0.72$
10^2
10^4
10^6
10^8
10^{10}
Viral load (copies/mL)

FIG. 4C

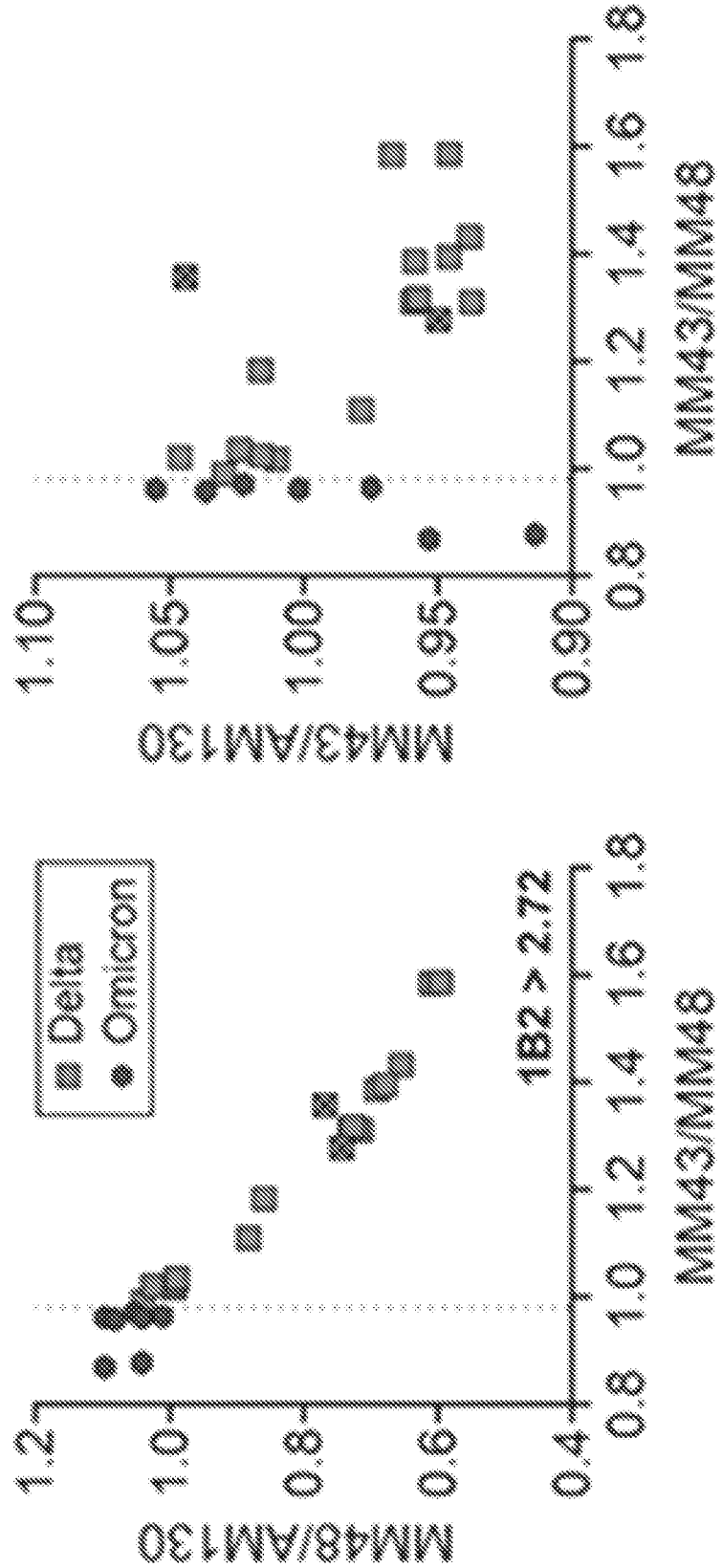
MM43/MM48
MM43/AM130
1.10
1.05
1.00
0.95
0.90
0.8
1.0
1.2
1.4
1.6
1.8
MM43/MM48
MM48/AM130
1.2
1.0
0.8
0.6
0.4
182 > 2.72
Delta
Omicron

FIG. 4D

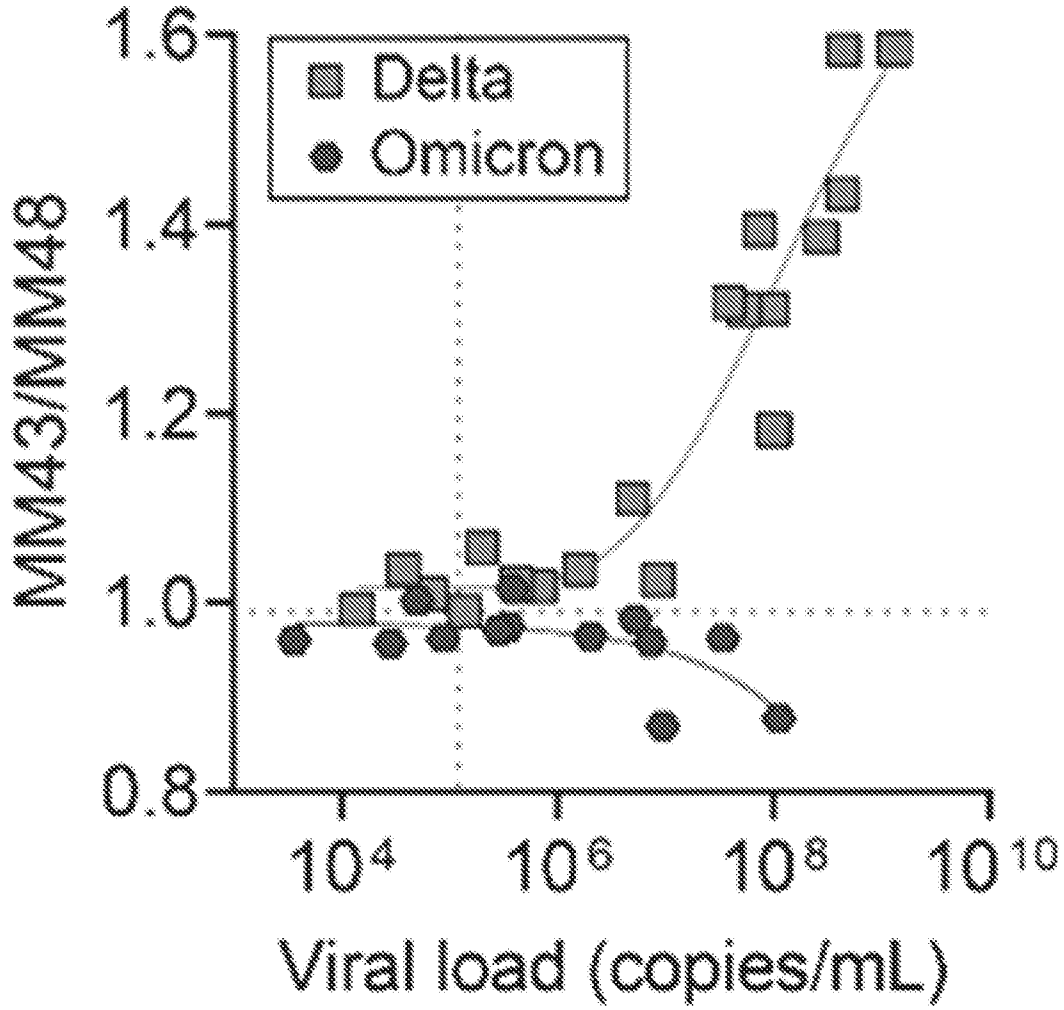
Delta
Omicron
MM43/MM48
1.6
1.4
1.2
1.0
0.8
10^4
10^6
10^8
10^{10}
Viral load (copies/mL)

FIG. 4E

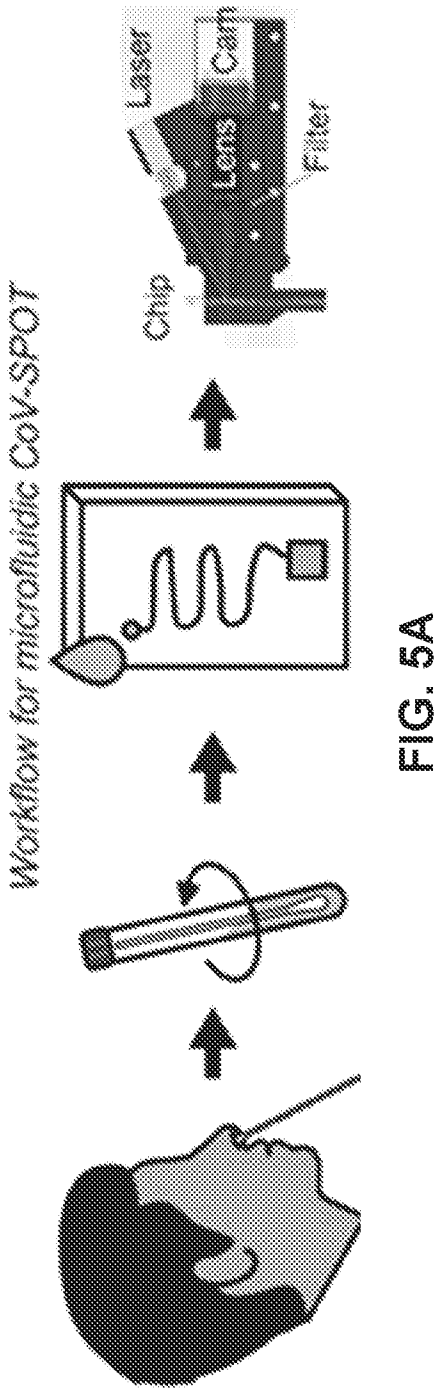
Workflow for microfluidic CoV-SPOT
Laser
Cam
Lens
Filter
Chip

FIG. 5A

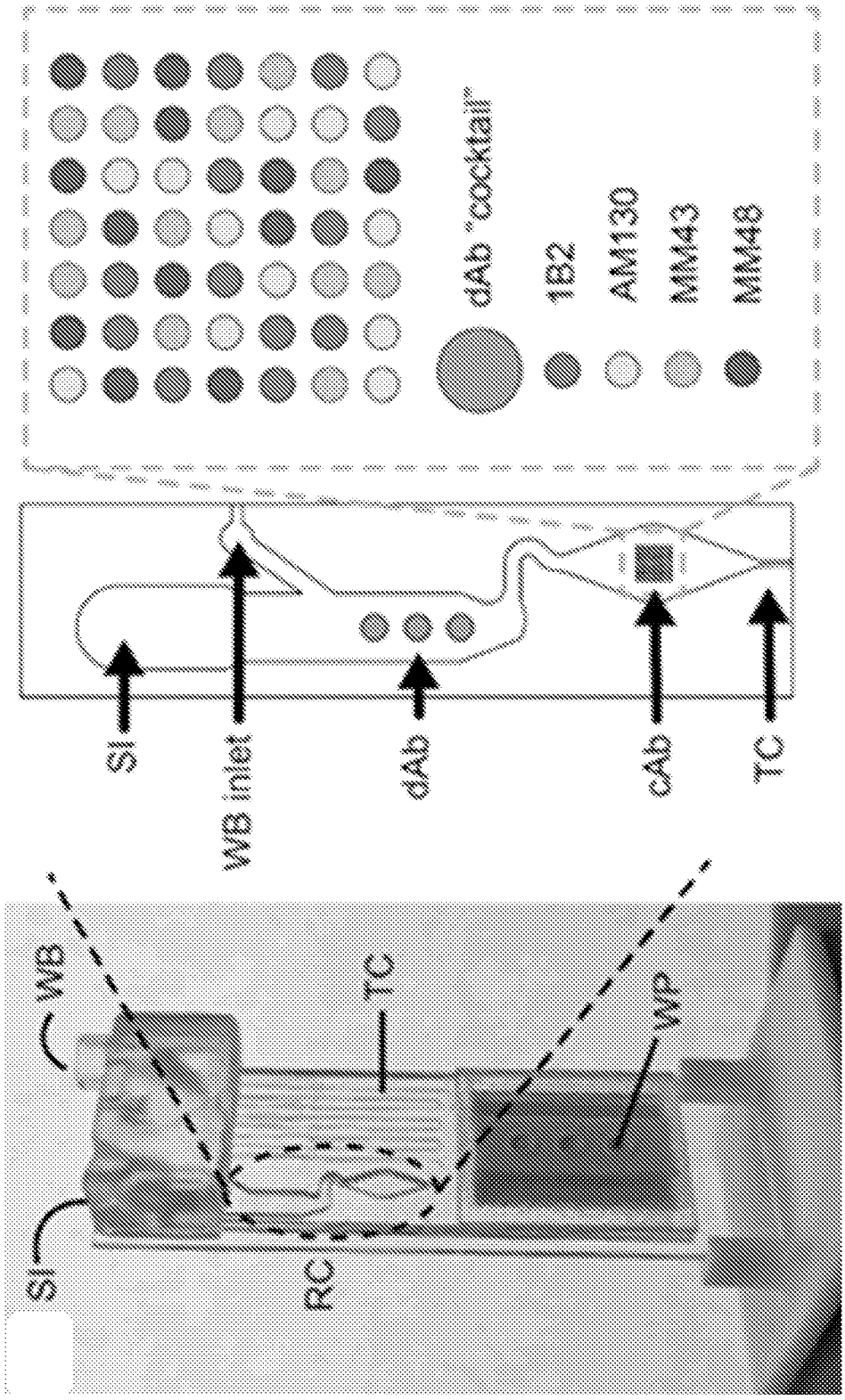
dAb "cocktail"
1B2
AM130
MM43
MM48
SI
WB inlet
dAb
cAb
TC
WB
TC
WP
SI
RC

FIG. 5B

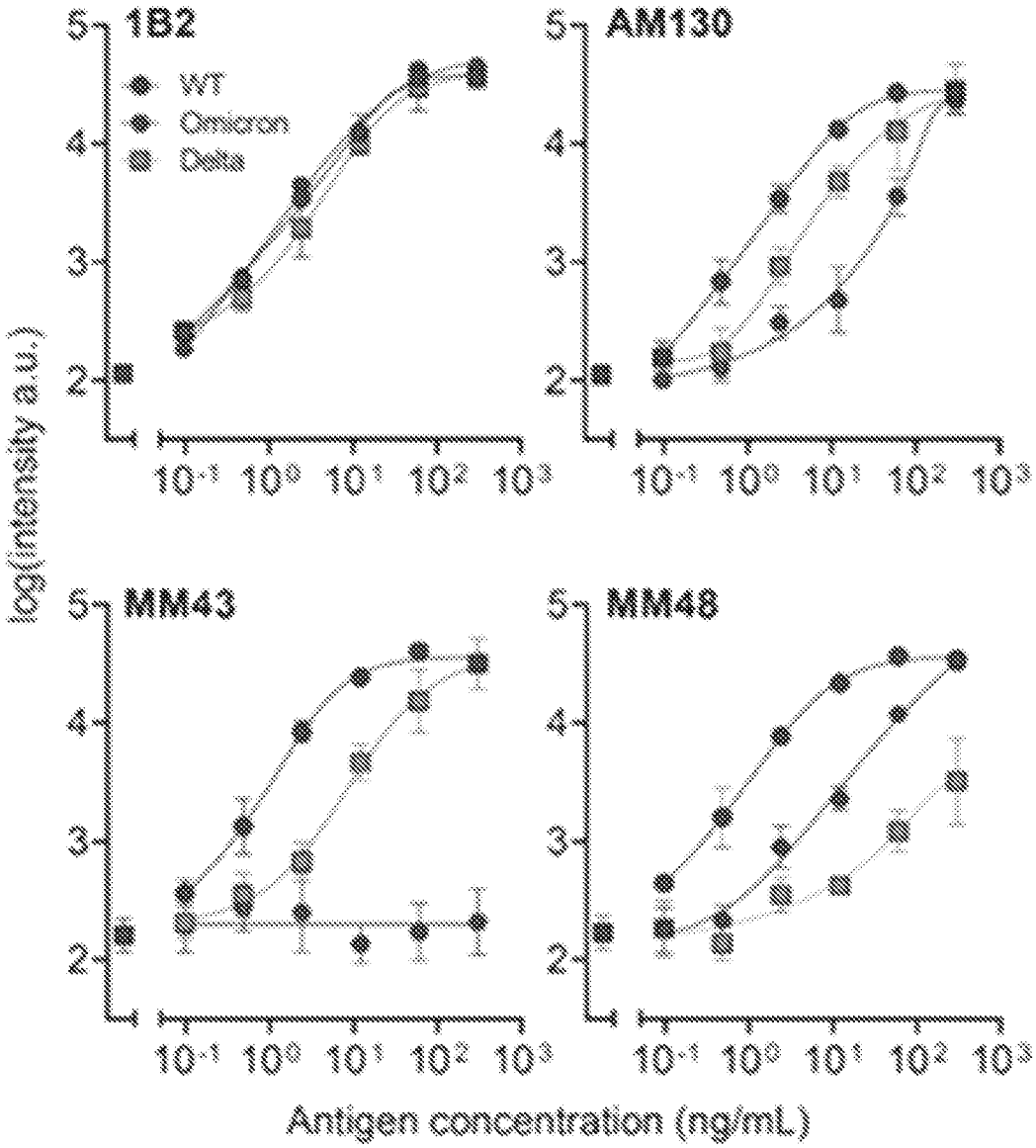
1B2
AM130
MM43
MM48
WT
Omicron
Delta
log(intensity a.u.)
Antigen concentration (ng/mL)

FIG. 5C

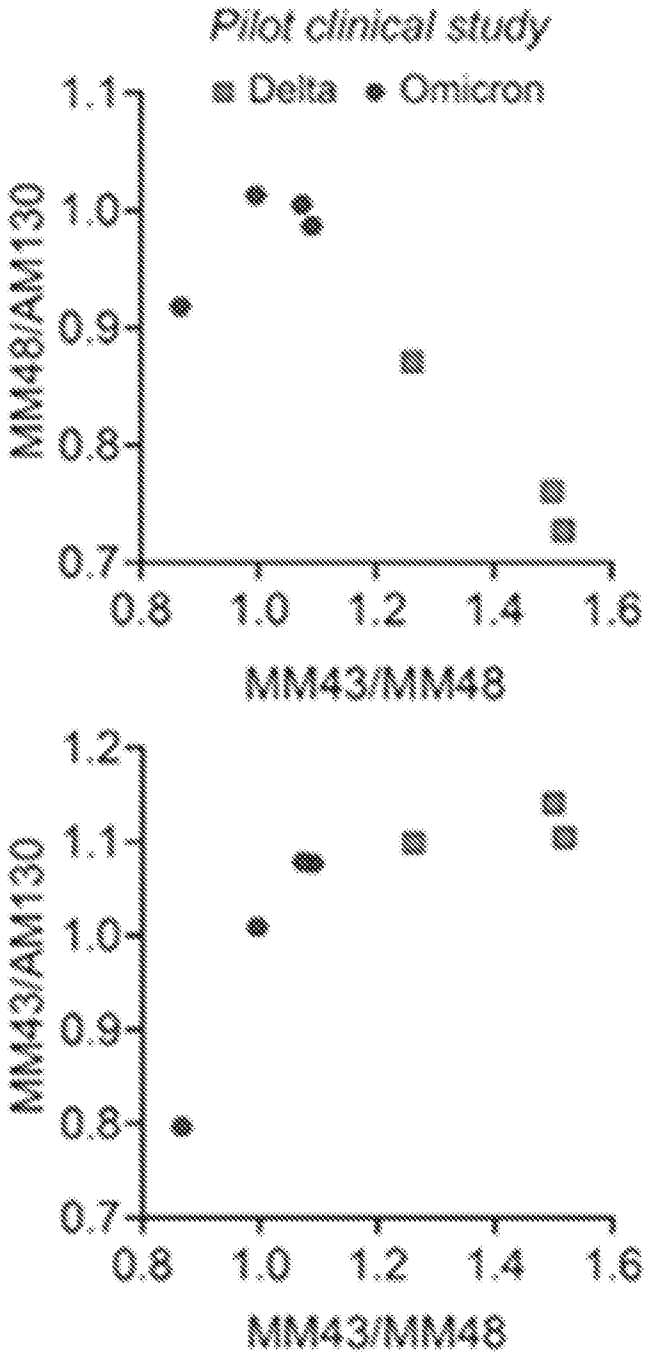
Pilot clinical study
Delta
Omicron
MM48/AM130
1.1
1.0
0.9
0.8
0.7
0.8
1.0
1.2
1.4
1.6
MM43/MM48
MM43/AM130
1.2
1.1
1.0
0.9
0.8
0.7
0.8
1.0
1.2
1.4
1.6
MM43/MM48

FIG. 5D

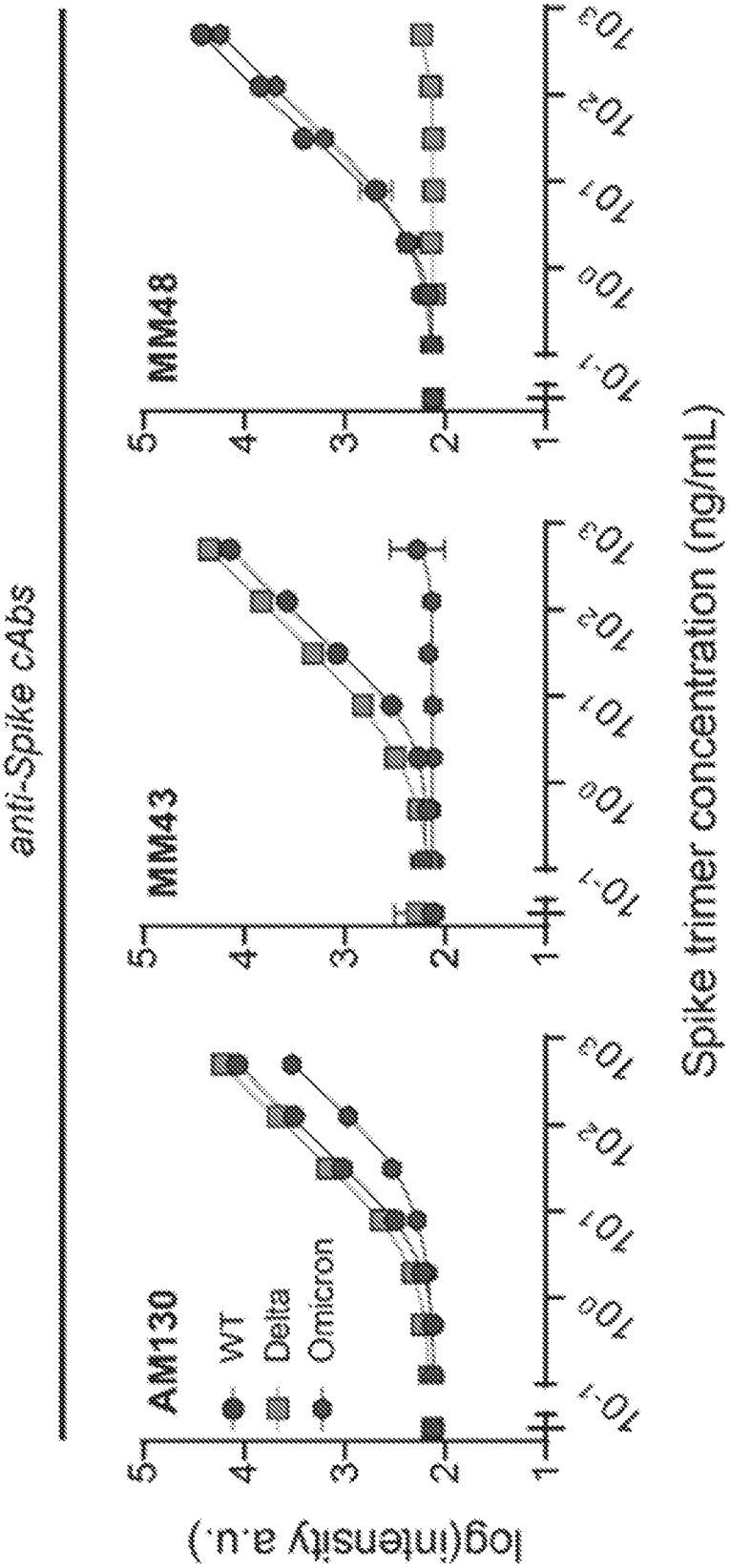
anti-Spike cAbs
AM130
MM43
MM48
WT
Delta
Omicron
log(intensity a.u.)
Spike trimer concentration (ng/mL)

FIG. 6A

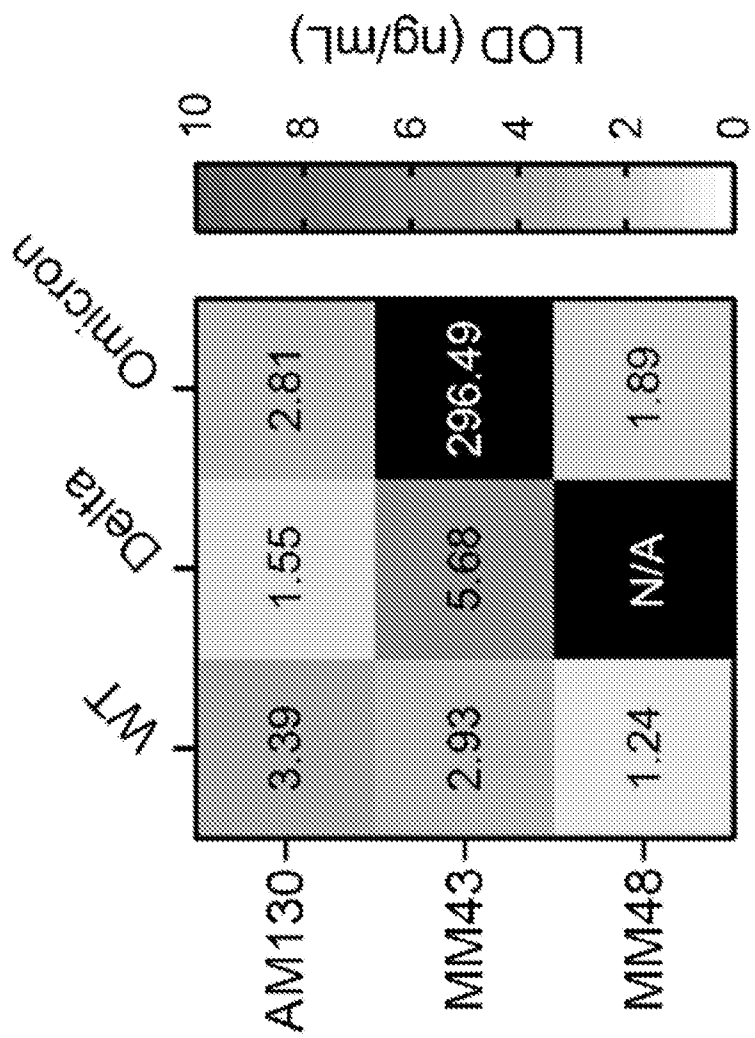
LOD (ng/mL)
10
8
6
4
2
0
Omicron
Delta
WT
AM130
MM43
MM48
3.39
1.55
2.81
2.93
5.68
296.49
1.24
N/A
1.89

FIG. 6B

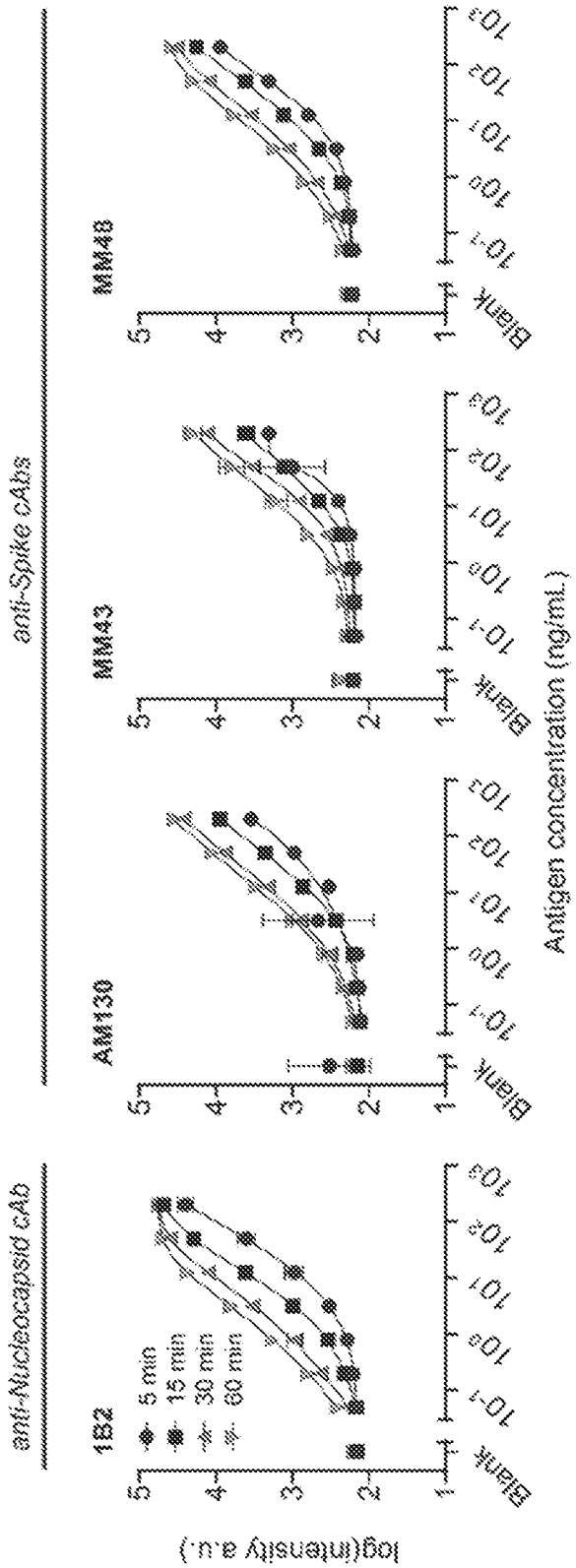
anti-Nucleocapsid cAb
anti-Spike cAbs
1B2
AM130
MM43
MM48
5 min
15 min
30 min
60 min
log(Intensity a.u.)
Antigen concentration (ng/mL)
Blank
10-1
100
101
102
103
1
2
3
4
5

FIG. 7

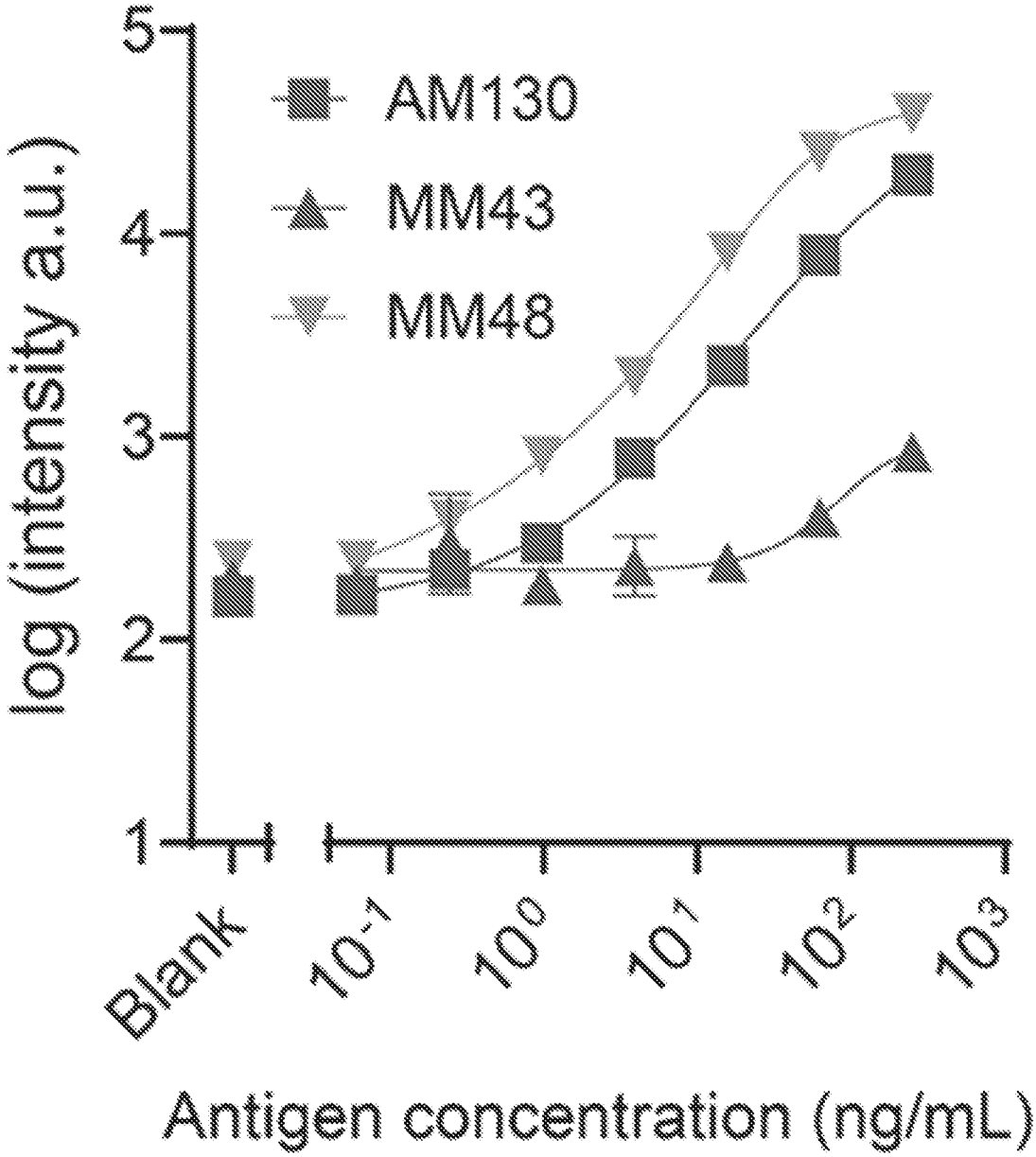
log (intensity a.u.)
5
4
3
2
1
AM130
MM43
MM48
Blank
10-1
100
101
102
103
Antigen concentration (ng/mL)

FIG. 8

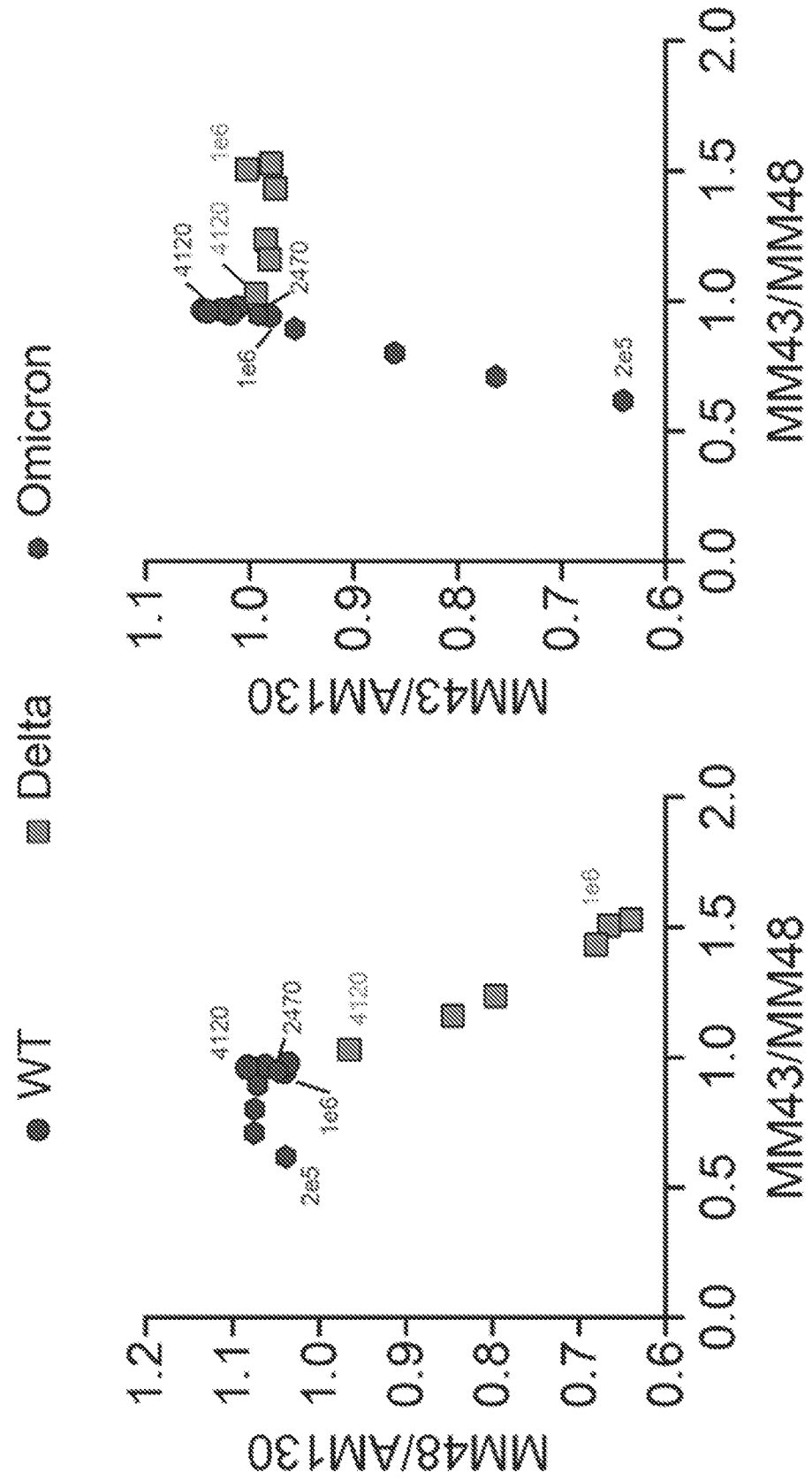
WT
Delta
Omicron
MM43/AM130
MM43/MM48
MM48/AM130
MM43/MM48
4120
2470
1e6
2e5
1e6

FIG. 9

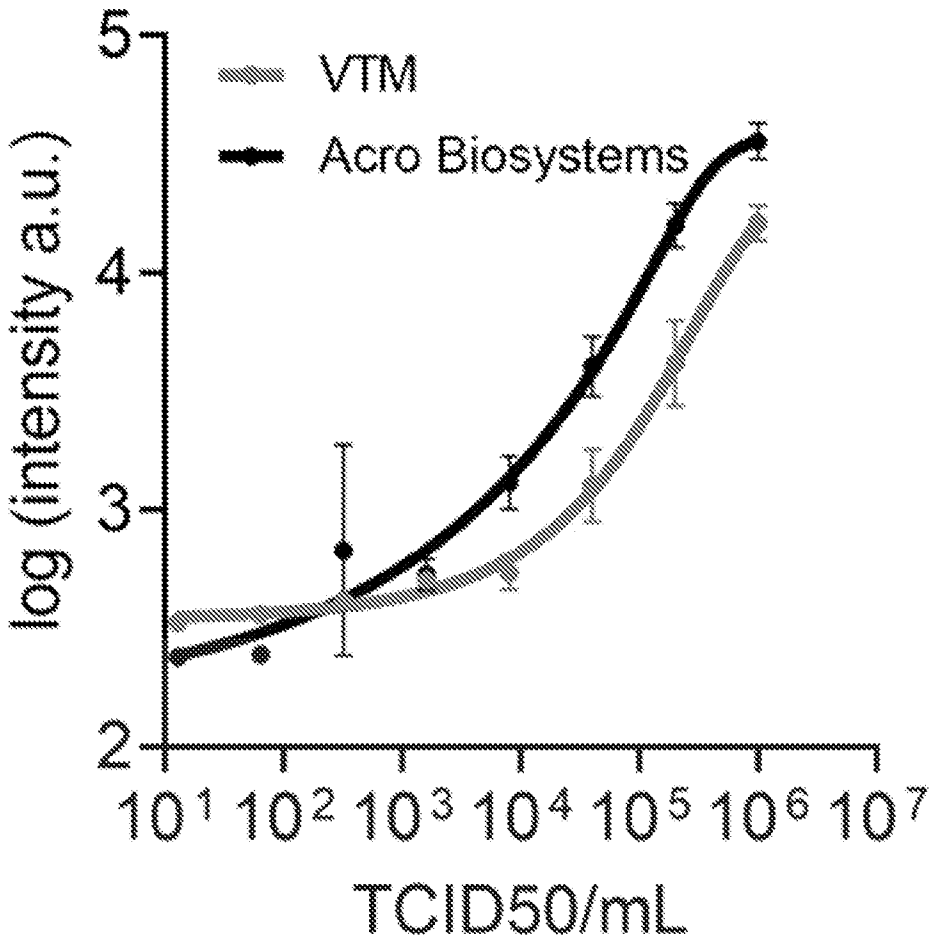
VTM
Acro Biosystems
log (intensity a.u.)
5
4
3
2
10^1 10^2 10^3 10^4 10^5 10^6 10^7
TCID50/mL

FIG. 10

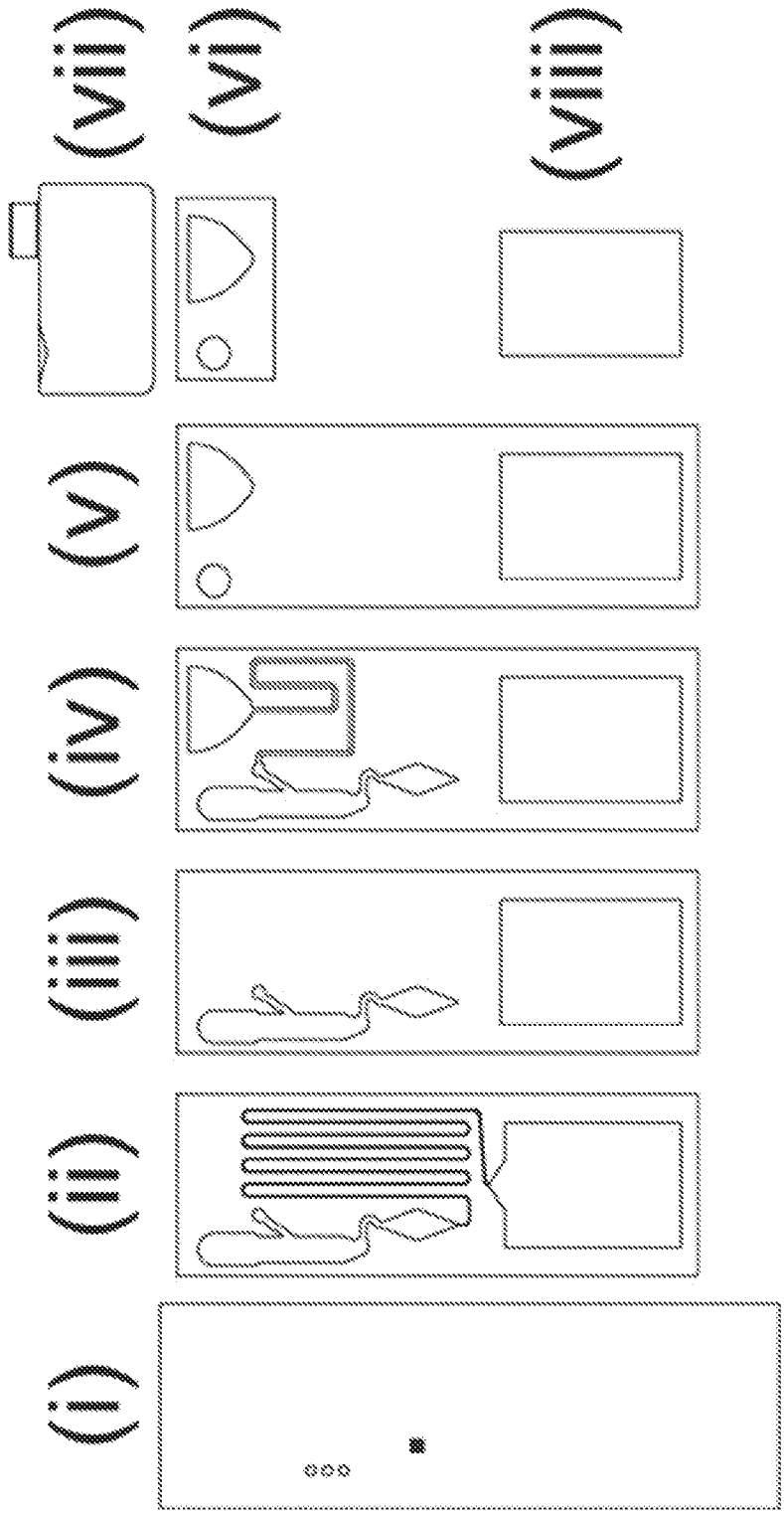

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/82105

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G01N 33/569, G01N 21/62 (2024.01)
ADD. G01N 33/68 (2024.01)

CPC - INV. G01N 33/56983, G01N 33/569, G01N 21/62
ADD. G01N 2470/06, G01N 33/68, G01N 2333/165, G01N 2469/10, G01N 2469/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEGGESTAD et al. "Rapid test to assess the escape of SARS-CoV-2 variants of concern"; Science Advances, Volume 7, Number 49 (December 2021), pg 1-12 (the entire document, and more specifically: pg 2, col 1, para 2; pg 8, col 2, para 3; figure 1A; abstract; title)	1-24
A	US 2022/0074938 A1 (SENSEUTICS LIMITED) 10 March 2022 (10.03.2022); the entire document, and more specifically: para [0002], [0011], [0074], [0137], [0224]-[0225]; figure 13; abstract; title	1-24
A	WO 2022/216821 A1 (HACKENSACK MERIDIAN HEALTH, INC.) 13 October 2022 (13.10.2022); the entire document	1-24
A, D	HEGGESTAD et al. "Multiplexed, quantitative serological profiling of COVID-19 from blood by a point-of-care test"; Science Advances, Volume 7, Number 26 (June 2021), pg 1-13 (the entire document)	1-24
A, P	MOHAMMAD et al. "Development and validation of a rapid and easy-to-perform point-of-care lateral flow immunoassay (LFIA) for the detection of SARS-CoV-2 spike protein"; Frontiers in Immunology, Volume 14 (February 2023), pg 1-15 (the entire document)	1-24
A, P	US 2023/0098149 A1 (DUKE UNIVERSITY) 30 March 2023 (30.03.2023); the entire document	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2024 (11.02.2024)	APR 10 2024
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300